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ENXAGUATÓRIOS BUCAIS A BASE DE ERVAS – EFEITO NO BIOFILME DENTAL, INFLAMAÇÃO GENGIVAL E HALITOSE: REVISÃO SISTEMÁTICA

Santa Maria, RS
2018

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, com ênfase em Periodontia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Ciências Odontológicas**.

Orientador: Prof. Dr. Fabricio Batistin Zanatta

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RESUMO

ENXAGUATÓRIOS BUCAIS A BASE DE ERVAS – EFEITO NO BIOFILME DENTAL, INFLAMAÇÃO GENGIVAL E HALITOSE: REVISÃO SISTEMÁTICA

AUTORA: Samantha Simoni Santi
ORIENTADOR: Fabricio Batistin Zanatta

Enxaguatórios bucais têm sido utilizados como coadjuvantes a higiene bucal na tentativa de otimizar o controle do biofilme dental, inflamação gengival e halitose. Neste contexto, a fitoterapia, com produtos a base de ervas e plantas, vêm ganhando uma maior aceitação mercadológica. A presente dissertação se propôs a revisar sistematicamente ensaios clínicos randomizados que avaliaram o efeito de enxaguatórios a base de produtos ervais, como coadjunvantes à higiene bucal. Foram realizadas 2 revisões sistemáticas sobre os desfechos de biofilme, inflamação gengival (RS1) e halitose (RS2). A busca foi conduzida nas bases de dados PubMed/MEDLINE, Cochrane - Central Register of Controlled Trials (CENTRAL), EMBASE, Web of Science, LILACS, Clinical Trials register, PROSPERO, The national dutch trial register, EU clinical trials register, NARCIS e literatura cinza *(opengrey*, banco de teses da capes) até 03 de abril de 2018. Não foi realizada restrição de língua. Na análise com desfechos de biofilme e inflamação gengival, 23 estudos foram incluídos e no desfecho halitose, 4 estudos foram incluídos. Devido à grande heterogeneidade observada nas características em ambos os estudos, não foi realizada meta-análise. Como resultados gerais, os enxaguatórios ervais demonstraram significativa redução em biofilme dental, inflamação gengival e halitose quando comparados às soluções placebo. No desfecho de biofilme e inflamação gengival as ervas *Camelia sinensis, Azadirachta indica, Anacardium occidentale Linn, Schinus terebinthifolius e Curcuma longa* mostraram resultados semelhantes à clorexidina. No desfecho halitose apenas um estudo comparou o produto fitoterápico (*Sesamum indicum*) com clorexidina, apresentanto resultados significativamente melhores para o produto erval no método auto-reportado. Entretanto, os estudos incluídos em ambas as revisões apresentaram, em sua maioria, risco moderado de viés, o que limita a validade interna das conclusões. Assim, ainda faltam evidências com menor risco de viés e que apontem mais claramente a magnitude do efeito dos enxaguatórios ervais para sua recomendação.

Palavras-chave: Antissépticos bucais. Fitoterapia. Gengivite. Halitose. Revisão sistemática.

ABSTRACT

HERBAL MOUTHRINSES – EFFECT ON DENTAL BIOFILM, GINGIVA INFLAMMATION AND HALITOSIS: A SYSTEMATIC REVIEW

AUTHOR: Samantha Simoni Santi
ADVISOR: Fabricio Batistin Zanatta

Mouthrinses have been used as adjuncts to oral hygiene in an attempt to optimize the control of dental biofilm, gingival inflammation and halitosis. In this context, herbal terapy, with products based on herbs and plants, have been gaining greater market acceptance. The present dissertation proposed to systematically review randomized clinical trials that evaluated the effect of herbal products as adjunct to oral hygiene. Two systematic reviews were made on dental biofilm, gingival inflammation (RS1) and halitosis (RS2) outcomes. The search was conducted in PubMed/MEDLINE, Cochrane - Central Register of Controlled Trials (CENTRAL), EMBASE, Web of Science, LILACS, Clinical Trials register, PROSPERO, The National Dutch Trial Register, EU clinical trials register, NARCIS and Gray literature (opengrey, thesis bank of capes) databases until April 3, 2018. No language restriction was performed. In the analysis with dental plaque and gingival inflammation outcomes, 23 studies were included and with halitosis outcome, 4 studies were included. Due to the high heterogeneity observed in the characteristics in both studies, no meta-analysis was performed. As a general result, herbal mouthrinses showed a significant reduction in dental dental plaque, gingival inflammation and halitosis when compared to placebo solutions. In the outcome of biofilm and gingival inflammation as well as some herbs *Camelia sinensis, Azadirachta indica, Anacardium occidentale Linn, Schinus terebinthifolius e Curcuma longa* results found for chlorhexidine. In the halitosis, only one study compared the herbal product (Sesamum indicum) with chlorhexidine, presenting significantly better results for the herbal product in the self-reported method. However, the studies included in both reviews presented, for the most part, a moderate risk of bias, which limits the internal validity of the conclusions. Thus, there is still a lack of evidence with less risk of bias and more clearly indicating the magnitude of the effect of herbal mouthrinse for its recommendation.

Keywords: Mouthrinse. Phytotherapy. Gingivitis. Halitosis. Systematic Review

SUMÁRIO

1 INTRODUÇÃO

O biofilme dental é formado por uma comunidade bacteriana que se estrutura a partir de um processo dinâmico de interações (MARSH et al., 2005). Quando acumulado na superfície dental, como conseqüência de falhas mecânicas na higiene, resulta no desenvolvimento de gengivite induzida por biofilme que ocorre em um período de 2-3 semanas; No entanto, está condição é reversível, sendo o reestabelecimento da saúde gengival alcançado após o retorno da higiene bucal adequada (LÖE; THEILADE; JENSEN, 1965; TROMBELLI et al., 2005).

A atual compreensão da patogênese das doenças periodontais indica que a presença de gengivite é um pré-requisito necessário para o desenvolvimento da periodontite (SANZ et al., 2011). Uma revisão sistemática recente levantou dados alarmantes, estimando a prevalência global de periodontite avançada em torno de 11%, com 743 milhões de pessoas afetadas em todo o mundo, constituindo a sexta doença mais prevalente no ser humano. Ainda, esta prevalência parece ter variações regionais, com o sul da América Latina e as regiões sub-saharianas do leste demonstrando prevalências em torno de 20% (KASSEBAUM et al, 2014).

Neste contexto, o controle mecânico da biofilme supragengival é parte fundamental na terapia periodontal (LÖE; THAILADE; JENSEN, 1965). Evidências longitudinais suportam o papel da gengivite como fator de risco para perda de inserção (SCHATZLE et al., 2003) e sugerem que estratégias de controle da biofilme supragengival, em nível populacional, podem reduzir não apenas a ocorrência de gengivite, mas também formas moderadas de periodontite (HUGOSON; SJODIN; NORDERYD, 2008).

No entanto, a prevalência quase onipresente de gengivite nas populações (ALBANDAR; KINGMAN, 1999; SUSIN et al., 2005) sugere falhas também quase onipresentes neste controle (RAMBERG; AXELSSON; LINDHE, 1995). Uma revisão sistemática sobre a efetividade dos autocuidados de higiene bucal por meio de escovação, de adultos com gengivite concluiu que a qualidade do controle mecânico de biofilmesupragengival não é suficiente para redução da gengivite (VAN DER WEIJDEN; HIOE, 2005).

Portanto, enxaguatórios bucais como adjuntos químicos tem sido cada vez mais indicados e estudados, com objetivo de melhorar o controle de biofilme e da inflamação gengival (BATTINO et al., 2002). Frente a isso, estudos com agentes

químicis à base de ervas medicinais vem ganhando uma posição de destaque, demonstrando que os extratos de plantas e seus compostos isolados apresentam um grande potencial antibacteriano contra vários patógenos orais (AMBROSIO et al., 2008; CUNHA et al., 2007; PORTO et al., 2009).

2 REVISÃO DE LITERATURA

2.1 AGENTES QUÍMICOS COMO COADJUVANTES À HIGIENE BUCAL

Em algumas situações temporárias e/ou permanentes o controle mecânico do biofilem supragengival pode estar comprometido. Nestes casos, pode-se lançar mão de produtos químicos como coadjuvantes ao controle mecânico caseiro (RÖSING, MALTZ; OPPERMANN, 2005).

Um exaguatório bucal ideal deve diminuir a carga e proliferação bacteriana na cavidade bucal, a adesão de microrganismos nas superfícies dentais, ser efetivo na redução de biofilme e inflamação gengival, ter sabor agradável e produzir mínimos ou nenhum efeito adverso (MOREIRA et al., 2009).

Anos de pesquisas documentadas estabeleceram que o digluconato de clorexidina (CHX) é seguro, estável e efetivo no controle do biofilme dental, sendo considerado o agente químico padrão-ouro dentre os enxaguatórios (LÖE et al., 1976, LANG; BRECX, 2006; GUNSOLLEY, 2010). A revisão sistemática de Van Strydonk et al., 2012, avaliou o uso de CHX na presença de higiene bucal auto realizada (HO) comparado com o uso de placebo mais HO em pacientes com gengivite. Os autores demonstraram que enxaguatórios de CHX usados como complemento à HO reduziram 33% [95% IC: 0.82 - 0.52] e 26% [95% IC: 0.42 - 0.23] a mais o biofilme acumulado e a inflamação gengival, respectivamente, que soluções placebo. A despeito desta superior ação anti-biofilme à curto prazo, uma revisão sistemática recente de ensaios clínicos randomizados (ECRs) com mais de 6 meses de duração indicou que ação da CHX na inflamação gengival não parece ser superior aquela alcaçada com produtos a base de Óleos Essenciais (EO) quando ambos são usados como complemento a higiene bucal mecânica (VAN LEEUWEN et al.,2011).

Apesar da CHX ser considerada o padrão ouro dos enxaguatórios bucais, seu uso à longo prazo é limitado pelos efeitos adversos, como manchamento dos dentes

e restaurações, alteração no paladar e aumento da formação de cálculo (LÖE et al., 1976, ADDY, 1979; VAN STRYDONCK et al, 2012). Por outro lado,o cloreto de cetilpiridínio (CCP), o triclosan e os óleos essenciais (EO) em uso prolongado apresentam também efeitos indesejáveis, como a sensação de ardência na língua e mucosas, (MARINHO; ARAÚJO, 2007; MOREIRA et al., 2009) manchas extrínsecas, devido à interação do CCP com corantes contidos nos alimentos (SHEEN; OWENS; ADDY, 2001), e aumento da formação de cálculo (GRANJEIRO et al., 1993).

Assim, o uso de alternativas naturais, como extratos de ervas e plantas, vem ganhando uma maior aceitação no mercado odontológico e resultando em um crescente interesse em pesquisas para novas formulações (KOTHIWALE et al., 2014).

2.2 HALITOSE

Halitose, malodor oral ou mau hálito são termos gerais usados para descrever um odor que emana da cavidade bucal. Aproximadamente 87% (DELANGHE et al., 1997) dos casos de mau hálito têm origem bucal, restando cerca de 5-13% atribuídos a causas não orais como sinusite crônica, diabetes mellitus, uremia e tonsilite crônica (MADHUSHANKARI et al., 2015; KAPOOR et al., 2016).

Evidências sugerem que a halitose é comum e pode afetar todas as idades. A grande maioria dos estudos relatam prevalências de cerca de 31,8% (SILVA et al.2017), mas alguns estudos estimam que mais de 50% da população tem halitose (BOLLEN; BEIKLER, 2012). A halitose frequentemente provoca constrangimento, podendo afetar a comunicação social interpessoal (DE GEEST et al., 2016). Neste sentido, produtos com propostas anti-halitose também se difundiram e se constituíram em um importante mercado para a indústria farmacológica e cosmética.

Compostos voláteis de enxofre (CVs) são os principais componentes responsáveis do malodor emanado do ambiente bucal. O sulfureto de hidrogênio (H_2S), o metilmercaptano (CH_4S) e o sulfureto de dimetilo $(CH_3O)_2SO_2)$ são os principais compostos envolvidos na halitose. Entretanto, o ácido propiônico, a putrescina e a cadaverina também estão secundariamente envolvidos (GOLDBERG et al.,1994). Esses compostos são produzidos pelo metabolismo da biofilme bacteriano presente em diferentes nichos bucais, degradação de resíduos

alimentares, células descamadas e proteínas salivares (LOESCHE; KAZOR, 2002).

A medição organoléptica (ORG) é considerada o padrão-ouro no diagnóstico da halitose. Na avaliação organoléptica, um “examinador treinado” cheira o ar expirado e avalia se é ou não agradável usando uma escala de intensidade, normalmente de 0 a 5 pontos (ROSENBERG et al., 1995). Entretanto, outros dispositivos podem ser utilizados para medição do mau odor, como o Halímeter (VSC), que analisa eletronicamente a concentração de sulfeto de hidrogênio e metilmercaptana, e, a cromatografia gasosa (CG), que mede as concentrações de três compostos importantes (sulfeto de hidrogênio, metilmercaptana e dimetilsulfeto) (ROSENBERG et al., 1991). Estes dispositivos eletrônicos têm sido utilizados e demonstraram ser reprodutíveis, embora, possivelmente menos confiáveis que os métodos organolépticos devido a não detectarem todos os CVs envolvidos com a halitose (YAEGAKI; COIL, 2000, KAPOOR et al., 2016).

O sucesso de qualquer intervenção para tratamento da halitoseestá na dependência, da eficácia na redução dos níveis de CVs. Assim, as intervenções são baseadas na redução da biofilme bacteriana presente nas superfícies dentárias e dorso da língua, tratamento das doenças periodontais e selamento de cavidades dentárias, juntamente com uma efetiva rotina de higiene oral do paciente (DEUTSCHER et al.,2017). O uso de nxaguatórios bucais contendo agentes antimicrobianos, tais como CHX, EO e cloreto de cetilpiridinio podem ser alternativas terapêuticas pois temum papel importante na redução dos níveis de bactérias produtoras de halitose. Já o dióxido de cloro e os sais de zinco podem ser eficazes na neutralização de compostos sulfurosos, mascarando temporariamente o mau odor (VAN DER SLEEN, 2010; BLOM et al., 2012). Porém, estes agentes estão associados com formulações alcoólicas e/ou outros ingredientes industriais. Assim, o uso de alternativas naturais tem recebido maior aceitação mercadológica (WILSON; DANISHEFSKY, 2006).

2.3 PRODUTOS ERVAIS

Os chamados medicamentos fitoterápicos são preparações vegetais padronizadas que consistem de uma mistura complexa de uma ou mais substâncias presentes em plantas, e posteriormente prescritos em obediência à legislação vigente (DI STASI, 2007). De modo geral, os compostos fitoterápicos podem ser

utilizados nas mais variadas fórmulas, como cápsulas, comprimidos, géis, pomadas, soluções aquosas, soluções hidroalcoólicas e infusões com atividades antissépticas, antibacterianas, antifúngicas, cicatrizantes e imunodepressoras (FRANCISCO, 2010).

A redução de custos e efeitos colaterais, em comparação às drogas convencionais, têm colocado os produtos fitoterápicos em lugar de destaque nos cuidados com a saúde nos últimos anos (BUCHAUL, 2001). Dentro dessa perspectiva, a participação das plantas medicinais no que tange à identificação e o desenvolvimento de novas moléculas ou protótipos básicos tem papel importante para geração de novos medicamentos, visto que muitos constituintes de plantas e/ou seus derivados semi-sintéticos constituem uma parcela apreciável dos novos medicamentos introduzidos no mercado (WILSON; DANISHEFSKY, 2006).

A Organização Mundial de Saúde (OMS) reconheceu em 1976 o potencial dos fitoterápicos (resolução da World Healtyh Assembly (WHA) 30.49). Em 1978, a resolução WHA 31.33 recomendou uma padronização das plantas medicinais com relação à identificação, purificação, produção, testes de segurança e eficácia, incentivando investimentos públicos em plantas medicinais. No Brasil, a fitoterapia faz parte do sistema biomédico de saúde desde 1995 e é regulamentada pela Agência Nacional de Vigilância Sanitária (ANVISA) desde 2000, a qual se baseia nos critérios de regulamentação Europeus. Na floresta amazônica há cerca de 55 mil espécies de plantas, das quais se estima que 10 mil possam apresentar propriedades medicinais (BARATA; QUEIROZ, 1995). Apesar dessa biodiversidade, o Brasil não se destaca no mercado mundial de fitoterápicos, mas vem desenvolvendo um crescente número de pesquisas nessa área (YUNES; PEDROSA; YURI, 2001; MANIPAL et al., 2016; DHINGRA; VANDANA, 2017). Em muitos países, a medicina complementar alternativa (MCA) está disponível há bastante tempo. Esses medicamentos geralmente são recomendados para condições não graves (COHEN; PAQUETTE; CAIRNS 2005, HARRINGTON; SHEPHERD, 2002). A MCA cresceu rapidamente nas útimas duas décadas. Em países de alta renda, há um crescente interesse popular por MCA. Nos Estados Unidos, por exemplo, aproximadamente 38% dos adultos e 12% das crianças estão usando alguma forma de MCA (EISENBERG et al.,1998)

Na odontologia, apesar do uso da fitoterapia ser milenar, a utilização de plantas medicinais para auxiliar no tratamento de doenças bucais ou de doenças

sistêmicas com manifestações bucais ainda é pouco explorada (VARONI et al., 2012). Algumas ervas têm sido aplicadas na terapia periodontal na busca de melhorar a eficácia do controle caseiro do biofilme supragengival e inflamação gengival (WILLERSHAUSEN;GRUBER; HAMM, 1994; KATSURA et al., 2001; LI; CAI; WU, 1997).

Dentre algumas opções de ervas utilizadas, encontram-se *Thymus vulgaris*, *Matricaria chamomilla*e e *Salvia officinalis,* as quais demonstraram propriedades antissépticas, adstringentes e desinfetantes (KOZEL, 1997) e estão disponíveis no mercado como o creme dental Sorriso Herbal® (Osasco, SP, Brasil). Outras ervas como o *Propolis wax* e a *Malva sylvestris,* com propriedades antimicrobianas, anti-inflamatórias, imunoestimulatórias, antitumorais e cicatrizantes (FIGUEIREDO et al., 1999) estão disponíveis comercialmente nos produtos Propamalva® (Ribeirão Preto, SP, Brasil) (xarope e *spray*) e Malvatricin® (Rio de Janeiro,RJ, Brasil), respectivamente.

Ainda, algumas ervas apresentam moléculas estruturais diversificadas, sendo denominadas terpenóides, glicosídeos, flavonóides e polifenóis, cuja atividade antimicrobiana não é significativa quando comparada a controles positivos. Entretanto, parece ocorrer um sinergismo entre estas moléculas, aumentando sua eficácia no controle de infecções (HEMAISWARYA; KRUTHIVENTI; DOBLE, 2008).

Posto isto, há uma série de produtos ervais cujas propriedades já foram avaliadas e seus efeitos clínicos testados. Considerando a importância do controle de biofilme supragengival, inflamação gengival e da halitose no contexto da terapia periodontal, esta dissertação objetivou revisar sistematicamente a literatura disponível para sumarizar o efeito de enxaguatórios bucais contendo produtos ervais nos desfechos de placa dental, inflamação gengival e halitose através de 2 revisões sistemáticas.

3 ARTIGO I – HERBAL MOUTHRINSES – EFFECT ON DENTAL PLAQUE AND GINGIVAL INFLAMMATION: A SYSTEMATIC REVIEW

Este artigo será submetido ao periódico *Journal of Clinical Periodontology*, ISSN: 1600-051X, Fator de impacto = 4.046; Qualis A1. As normas para publicação estão descritas no Anexo A.

Herbal mouthrinses – Effect on dental plaque and gingival inflammation: a systematic review

Running Title: Herbal mouthrinses on plaque and gingivitis

KEY WORDS: mouthrinse, phytotherapy, dental plaque, clinical trial, systematic review

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Clinical Relevance

Scientific rationale for the study: The effect of mouthrinses containing herbal products on dental plaque and gingival inflammation remain controversial. Therefore, a systematic review of randomized clinical trials was conducted.

Principal findings: Significant differences were found favoring herbal mouthrinses over placebo solutions for all measures of dental plaque and gingival inflammation *Camelia sinensis, Azadirachta indica, Anacardium occidentale* Linn, *Schinus terebinthifolius* and *Curcuma longa* demonstrated equivalent results than Chlorhexidine. However, the quality of the most studies were moderate.

Practical implications: Herbal mouthrinses as adjuvants to mechanical control are promising, but randomized clinical trials of better quality should be conducted.

Abstract

Aim: Mouthrinses based on herbs and plants have been gaining greater market acceptance and research. Therefore, the aim of the present study was to perform a systematic review of randomized clinical trials evaluating the effect of herbal mouthrinses as an adjuvant to oral hygiene on dental plaque and gingival inflammation in patients with gingivitis.

Material and Methods: Searches were conducted in the PubMed / MEDLINE, Cochrane - CENTRAL, EMBASE, Web of Science, LILACS / BIREME, Clinical Trials Registryand grey literature for articles published up to April, 2018. No language restriction was imposed.

Results: From 4013 paper found, 23 studies met the eligibility criteria and were included. Meta-analysis was not possible due to the considerable heterogeneity of the studies. The herbal mouthrinses achieved significant reductions in dental plaque and gingival inflammation compared to placebo solutions. Moreover, *Camelia sinensis, Azadirachta indica, Anacardium occidentale* Linn, *Schinus terebinthifolius* and *Curcuma longa*, proved to be promising when compared to chlorhexidine 0.2% and 0.12%.

Conclusion: The methodological quality of the majority of studies analyzed revealed a moderate risk of bias. Despite the promising results, there is a need for evidence that indicate the effect size of herbal mouthrinses and their clinical relevance. PROSPERO registry number: CRD42015016081

Introduction

In the 1960s, when Löe and colleagues established the unequivocal role of dental plaque as the etiological agent of gingivitis, mechanical plaque control became the basis of periodontal therapy (Löe, Theilade, & Jensen, 1965). However, the nearly omnipresent prevalence of gingivitis suggests that plaque control by mechanical means is inefficient, since patient efforts are often compromised by areas of difficult access, inadequate ability and low motivation (Osso & Kanani, 2013; Van der Weijden & Hioe, 2005). This difficulty in obtaining "ideal" plaque control has led scientists and clinicians to seek antimicrobial chemical agents that could help inhibit the formation of plaque on the surfaces of the teeth (Gunsolley, 2010; Stoeken, Paraskevas, & Van der Weijden, 2007; Van Strydonck, Slot, Van Der Velden, & Van Der Weijden, 2012).

The use of antimicrobial agents may be beneficial to adjunct supragingival plaque control (Barnett, 2006; Escribano et al., 2016; Serrano, Escribano, Roldan, Martin, & Herrera, 2015). Currently, a wide range of commercial products is available in the form of toothpastes and mouthrinses to control the growth of dental plaque and gingival inflammation. However, many of these formulations contain alcohol, that the long-term adverse effects are not know (Cole, Rodu, & Mathisen, 2003; Satpathy et al., 2013; Zamora-Perez et al., 2013), and some agents, such as chlorhexidine, have several adverse effects (Van Strydonck, Slot, Van Der Velden, & Van Der Weijden, 2012.

Reductions in costs and side effects compared to conventional drugs have placed herbal products in a prominent position in health care in recent years (Eisenberg et al., 1998; Varoni, Lodi, Sardella, Carrassi, & Iriti, 2012). In 1977, the World Health Assembly (WHA) of the World Health Organization (WHO) recognized the potential of herbal medicines (WHO, 1977). In 1978, the WHO recommended the standardization of medicinal plants for identification, purification, production, safety and efficacy testing, encouraging public investments in medicinal plants (WHO, 1978).

The use of medicinal plants for the treatment of oral diseases or systemic diseases with oral manifestations has been very exploired. However, there are controversial results related to the effects of oral herbal mouthrinses as an adjuvant to oral hygiene in individuals with gingivitis. Moreover, no systematic review was performed addressing this issue. Thus, the aim of the present study was to perform a

systematic review of randomized clinical trials to determine the effect of herbal mouthrinses as an adjuvant to oral hygiene compared to a placebo, positive control or mechanical oral hygiene alone to reducing dental plaque and gingival inflammation in adults with gingivitis.

Material and Methods

This study was conducted in accordance with the Cochrane Handbook (Higgins & Green, 2011) and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA statement) (Moher et al., 2009). The protocol is registered with the International Prospective Register of Systematic Review (PROSPERO) – CRD42015016081.

Focused question (PICOS)

Do mouthrinses with herbal products adjunct to mechanical oral hygiene have a effect on plaque and gingival inflammation compared to a placebo rinse, control rinse or regular mechanical oral hygiene when used by healthy adults with gingivitis for at least one week in randomized controlled clinical trials?

As a secondary outcome measure, potential side effects were investigated.

Search strategy

Searches were conducted in the US National Library of Medicine databases (PubMed / MEDLINE), Cochrane - Central Register of Controlled Trials (CENTRAL), EMBASE, Web of Science, Latin American and Caribbean Center on Health Sciences (LILACS / BIREME), Clinical Trials Registry, PROSPERO, The National Dental Trial Registry, US Clinical Trials Registry and grey literature (OpenGrey, CAPES thesis bank) for articles published up to April, 2018.

The search in PubMed/MEDLINE was performed using a combination of controlled vocabulary and keywords (Table 1). A similar search strategy was adapted for the other databases. No restriction was imposed with regard to language. The Mendeley Desktop 1.17.13 reference manager (England) was used to group and manage the references.

Eligibility criteria

- Study design: Randomized controlled clinical trials with no language restriction;
- Participants: healthy adults, ≥ 18 years old, with diagnosis of gingivitis;

- Intervention: An herbal mouthrinse used for at least one week;
- Comparison: Placebo rinse, control rinse or regular oral hygiene;
- Outcomes: Dental plaque and/or gingival inflammation.

Screening and selection

Two trained reviewers (MC and APG) independently screened all titles and abstracts for eligible papers. Papers without abstracts, but with titles suggesting a relation to the objectives of the review were also pre-selected and submitted to the full-text analysis for eligibility. The full texts of the pre-selected articles were read in detail by the same reviewers (MC and APG) (*Kappa*= 0.93).

Papers that fulfilled all of the selection criteria were processed for data extraction (*K*= 0.89). Duplicates were excluded. Studies that fulfilled the eligibility criteria were included in the present review. Divergences of opinion between the reviewers were resolved by a discussion. If a disagreement persisted, the judgment of a third reviewer (FBZ) was considered decisive. When necessary, the authors of the selected articles were contacted to clarify any ambiguities and provide missing data.

Data extraction

The following data were extracted: (1) data from the study: (A) authors, year of publication and country; (B) design (parallel or cross-over) and duration of follow up. (2) intervention and control: (A) scientific name, common name, brand name and formulation of the produts used. (3) protocol: prophylaxis prior to onset of experiment, hygiene instructions during study, dose (milliliters [mL]), number of times per day, time of use, and use of oral hygiene adjuvant. (4) participants: (A) sample size; (B) age; (C) sex; (D) inclusion and exclusion criteria employed. (5) Results: (A) evaluation criteria for gingival inflammation and dental plaque. (B) measures at baseline and end of study. In cases of missing data, e-mails were sent to the authors for clarification. If no response was obtained, the data were extracted from graphs and figures with the aid of the WebPlotDigitizer 3.8 (Copyright ©, Austin, Texas, USA). Information on variance were retrieved with the aid of the R software, version 3.4.1 (Copyright©, Vienna, Austria, 2017) or imputed in a conservative manner (MacKinnon, 2010).

Data analysis

A preliminary assessment of the selected papers revealed considerable clinical and methodological heterogeneity among the studies. The main differences were related to the herbal products used and methodological characteristics (population studied, interventions, comparison procedures and evaluation period), rendering meta-analysis of the data impossible. Therefore, the findings are presented in a descriptive manner.

Risk of bias (quality) assessment

The quality of the studies included in the present review was appraised by two trainedreviewers (APG and MC) independentky screened (K = 0.98), using a bias appraisal method specific to the study design (Cochrane Handbook for Systematic Reviews of Interventions 5.0.1; Higgins & Green 2011). The criteria were adapted and divided into seven principal domains. For each study, the criteria were classified as exhibiting a low, high or unclear (without information or uncertain) risk of bias. For the final classification of the risk of bias, divergences of opinion between the reviewers were resolved by consensus.

Results

Search and selection results

The searches on the electronic databases led to the retrieval of 4013 studies. After the analysis of the titles and abstract, 262 studies were pre-selected for the full-text analysis. Following the application of the eligibility criteria, 239 of these studies were excluded and 23 were included in the present review: 20 from MEDLINE/Pubmed, two from EMBASE and one from Clinical Trials Registry. The reasons for the exclusions are detailed in Fig. 1.

Assessment of heterogeneity

Table 2 displays detailed information on the characteristics of the studies. Considerable heterogeneity was found in the duration (evaluation period), mouthrinse protocol and herbal products. Among the 23 clinical trials, only Gomes et al. (2016) employed a crossover design. The other 22 studies had a parallel design. Jayakumar et al. (2012) had the shortest duration period (seven days) and Grossman et al. (1989) had the longest (six months).

Considerable clinical heterogeneity was also found in the interventions. The herbal products used as an intervention involved 18 different types of plants. The following products and plants were used: Triphala® (Baratakke et al., 2017; Pradeep et al., 2016) Hiora® (Bhat et al., 2014; Bhate et al., 2015), *Terminalia chebula* (Gupta et al., 2014b; Gupta et al., 2015) *Camellia sinensis* (green tea) (Balappanavar et al., 2013; Priya et al., 2015; Rassameemasmaun et al., 2013) *Ocimum gratissimum* (Gupta et al., 2014a; Kothiwale et al., 2014; Pereira et al., 2011), sanguinarine (Veadent®) (Grossman et al., 1989), *Melaleuca alternifolia* (Kothiwale et al., 2014; Lauten et al., 2005; Saxer et al., 2003), *Cinnamomum zeylanicum* (Gupta & Jain, 2015), *Calendula* flower (Lauten et al., 2005; Mahyari et al., 2016), *Anacardium occidentale* Linn (Gomes et al., 2016)*, Verbena officinalis Linn* (Grawish et al., 2016), *Zingiber officinale, Rosmarinus officinalis* (Mahyari et al., 2016), *Cymbopogan citrates* (lemon grass) (Dany et al., 2015), *Matricaria chamomilla, Schinus terebinthifolius* (Lins et al., 2013), *Aloe vera* (Jayakumar et al., 2012) and *Curcuma longa* (Waghmare et al., 2011).

Fifteen studies performed comparisons of three different mouthrinses (Balappanavar et al., 2013; Baratakke et al., 2017; Bhat et al., 2014; Dany et al., 2015; Gomes et al., 2016; Gupta et al., 2014a; Gupta et al., 2014b; Gupta et al., 2015; Gupta & Jain, 2015; Jayakumar et al., 2012; Lauten et al., 2005; Lins et al., 2013; Mahyari et al., 2016; Pereira et al., 2011; Pradeep et al., 2016), comparing the different herbal products to chlorhexidine 0.2% or 0.12% and a placebo rinse or two groups containing herbal products compared to chlorhexidine. Seven studies (Bhate et al., 2015; Grawish et al., 2016; Kothiwale et al., 2014; Priya et al., 2015; Rassameemasmaung et al., 2013; Saxer et al., 2003; Waghmare et al., 2011) presented two different mouthrinses, comparing an herbal group to a placebo, chlorhexidine or essential oils (Listerine®). Only Grossman et al. (1989) investigated four different types of mouthrinse, a herbal product, chlorhexidine 0.12%, essential oils (Listerine®) and a placebo rinse

Regarding the mouthrinse protocol, the majority of studies indicated use twice a day. Only Saxer et al. (2003) employed a frequency of three times a day. All studies used 10 or 15 mL and rinsing time ranged from 30 to 90 seconds. The composition of each herbal product also varied. Some had the addition of alcohol, mineral water, distilled water and other substances.

Side effects

Five studies (Balappanavar et al., 2013; Baratakke et al., 2017; Bhat et al., 2014; Grawish et al., 2016; Pryia et al., 2015) investigated the side effects of the herbal products with the use of questionnaires, self-reports and a clinical evaluation of the participants. In the study conducted by Bhat et al. (2014), the participants reported a mild burning sensation with the use of the herbal product. Balappanavar et al. (2013) performeda questionnaire to the participants evaluate the mouthrinse and concluded that the herbal products employed did not have side effects. Grawish et al. (2016) who evaluated Verbena officinalis Linn and placebo, investigated tooth coloration, tongue coloration, burning sensation, altered taste perception and dry mouth and found no side effects with the use of the herbal product.

Prophylaxis and oral hygiene instructions

In eight studies (Dany et al., 2015; Grossman et al., 1989; Gupta et al., 2015; Jayakumar et al., 2012; Kothiwale et al., 2014; Lins et al., 2013; Pereira et al., 2011; Pradeep et al., 2016), the participants received oral prophylaxis at baseline. In ten studies (Balappanavar et al., 2013; Bhate et al., 2015; Grawish et al., 2016; Gupta & Jain, 2015; Kothiwale et al., 2014; Lins et al., 2013; Pereira et al., 2011; Priya et al., 2015; Rassameemasmaung et al., 2013; Waghmare et al., 2011), hygiene instructions were given and oral hygiene products were made available for oral home care. In seven studies, prophylaxis and oral hygiene instructions were not performed (Jayakumar et al., 2012; Bhat et al., 2014; Gupta et al., 2014b; Gupta et al., 2015 Gupta et al., 2014a; Lauten et al., 2005; Saxer et al., 2003).

Evaluation of outcomes

Table 3 displays detailed information on the assessment criteria for gingival inflammation, dental plaque as well as the baseline and final measures.

All studies found significant differences favoring the herbal products when compared to placebos in both outcome, plaque index and gingival inflammation. In comparison to chlorhexidine, while Bhate et al. (2015), Pereira et al. (2011) and Grossman et al. (1989) found significant reductions in dental plaque and gingival inflammation favoring chlorhexidine 0.12%, other authors did not find significant differences with regard to either outcome (Balappanavar et al., 2013; Baratakke et al., 2017; Bhat et al., 2014; Dany et al., 2015; Gomes et al., 2016; Grawish et al., 2016; Gupta et al., 2014a; Gupta et al., 2014b; Gupta et al., 2015; Jayakumar et al., 2012; Lauten et al., 2005; Mahyari et al., 2016; Pradeep et al., 2016; Priya et al.,

2015; Rassameemasmaung et al., 2013; Saxer et al., 2003; Waghmare et al., 2011; Kothiwale et al., 2014).

Six studies (Gomes et al., 2016; Gupta et al., 2014b; Balappanavar et al., 2013; Lins et al., 2013; Bhat et al.,2014; Prya et al., 2015) found percent reductions higher than CHX, favoring the herbal product for the outcome of dental plaque. Balappanavar et al. (2013) found significant reductions in dental plaque favoring the herbal product tested [*Camellia sinensis* (green tea)] over chlorhexidine 0.2%. Moreover, nine studies (Baratakke et al., 2017; Gomes et al., 2016; Dany et al., 2015; Gupta, 2014; Balappanavar et al., 2013; Lins et al., 2013; Jayakumar et al., 2012; Waghmare et al., 2011; Gupta el al.,2014a). found a higher percentage reduction in gingival inflammation favoring the herbal products over chlorhexidine.

Kothiwale et al. (2014) found favorable results with the use of essential oils (Listerine®) in relation to dental plaque and gingival inflammation compared to the tested herbal product (25% Melaleuca alternifolia, Syzygium aromaticum and Ocium basilicum), but without statistical differences.In contrast, Grossman et al. (1989) found significantly better results with the use of chlorhexidine 0.12% and Listerine® compared to the herbal product tested [0.03% sanguinarine extract and zinc (Veadent®)] and placebo.

Quality appraisal

Figure 2 displays detailed information on the methodological quality and risk of bias of the studies included in the present review. The risk of bias was uncertain in the majority of studies. A lmoderate risk of bias was found with regard to the blinding of the participants (50% of studies) and blinding of the examiners (60.7%). Five studies (Bhat et al., 2014; Bhate et al., 2015; Kothiwale et al., 2014; Lauten et al., 2005; Saxer et al., 2003) exhibited a high risk of attrition bias. More than 75% of the studies exhibited an uncertain risk with regard to allocation concealment and selective reporting of the outcome.

Discussion

The main findings of the present review indicate that the majority of herbal products led to significant reductions in dental plaque and gingival inflammation, with results very similar and even, in some cases, equivalent to those achieved with chlorhexidine.

The study of different herbal products is important in dental practice, some herbs have been applied in periodontal therapy in the search to improve the effectiveness of home control of supragingival plaque and gingival inflammation (Li; Cai, Wu, 1997; Katsura et al., 2001; Malhotra et al., 2011; Varoni et al., 2012). There is increasing public interest in phytotherapy over the past two decades (Nogales-Gaete, 2004). This increase can be justify because most of commercial mouthrinses have formulations with alcohol and other industrial ingredients for which the long-term adverse effects are not known (Loe, Schiott, Karring, & Karring, 1976; Van Strydonck, Slot, Van der Velden, & Van der Weijden, 2012). A systematic review showed association between an increased risk of head and neck cancer and the excessive, highly frequent or prolonged use of mouthrinses with alcohol (Boffetta et al., 2016). As confounding factors and other forms of bias could not be excluded, these findings are not definitive, but also cannot be ignored.

A systematic reviews have demonstrated the anti-plaque and anti-gingival inflammation effects of chlorhexidine and essential oils as an adjuvant to oral hygiene [33% and 26% greater reductions in dental plaque and gingival inflammation for chlorhexidine compared to a placebo solution (Van Strydonk et al., 2012) and 32% and 24% greater reductions for essential oils (Haas et al., 2016). Considering the effect size, the present findings demonstrate that some herbal products achieve similar or even superior results compared to chlorhexidine and essential oils.

With regard to gingival inflammation, nine studies with eleven herbs demonstrated similar effects to those achieved with chlorhexidine: *Emblica officinalis*, *Terminalia chebula*, *Terminalia belerica* (Triphala®), *Anacardium occidentale* Linn, *Cymbopogan citratus, Ocimum gratissimum, Azadirachta indica, Camellia sinensis, Schinus terebinthifolius, Aloe vera and Curcuma* longa (Baratakke et al., 2017; Gomes et al., 2016; Dany et al., 2015; Gupta, 2014; Balappanavar et al., 2013; Lins et al., 2013; Jayakumar et al., 2012; Waghmare et al., 2011; Gupta el al.,2014a). Five of these herbs (Gomes et al., 2016; Waghmare et al., 2011; Balappanavar et al., 2013; Lins et al., 2013) stood out for achieving a more than 50% reduction in gingival inflammation. Similar effects on this outcome compared to chlorhexidine were found for solutions with *Anacardium occidentale* Linn (56.6%) (Gomes et al., 2016), *Curcuma longa* (60.7%) (Waghmare et al., 2011), *Azadirachta indica* (55.6%),*Camellia sinensis* (54.5%) (Balappanavar et al., 2013), *Schinus terebinthifolius* (63.4%) (Lins et al., 2013). This anti-inflammatory effect may be due

to the inhibitory influence of these herbs on the synthesis of prostaglandins, which play a key role in inflammatory events (Ivan, 1999; Silva et al., 2012).

Green tea (*Camellia sinensis*) led to a greater percentage reduction in dental plaque compared to chlorhexidine (Balappanavar et al., 2013; Priya et al., 2015). However, Balappanavar et al. (2013) employed different periods of use for the products (two weeks for chlorhexidine and three weeks for green tea); this methodological difference may partially explain the differences in the effect of the herbal product. Priya et al. (2015) and Rassameemasmaung et al. (2013) also used *Camellia sinensis* (green tea) and found a significant ($P < 0.05$) reduction in gingival inflammation compared to chlorhexidine and a placebo, respectively. It should be pointed out that both studies had a moderate risk of bias, particularly with regard to blinding the participants and the selective reporting of the outcomes.

Camellia sinensis (green tea) is quite popular and *in vitro* evidence has shown that its polyphenols inhibit the growth and cellular adherence of periodontal pathogens as well as their production of virulence factors (Sakanaka et al., 1996; Hirasawa et al., 2002). Kushiyama et al. (2009) found that the ingestion of green tea was correlated with mean probing depth (PD), clinical attachment loss (CAL), and bleeding on probing (BOP). Each cup of green tea per day was associated with a 0.023 mm reduction in mean PD ($P < 0.05$), 0.028 mm reduction in mean CAL ($P < 0.05$) and 0.63% reduction in BOP ($P < 0.05$).

Most randomized clinical trials included in this review had short to intermediate duration (seven days to three months). Only Grossman et al. (1989) conducted an analysis for six months. Although mouthrinses are used and prescribed for short periods, the American Dental Association (ADA, 2008) stipulates a six-month study period for the evaluation of the efficacy and safety of mouthrinses. Due to the heterogeneity in the studies, it was not possible to determine if the time of use was directly related to the size of the effect of the herbal. In addition, there is no dosage and time of action in the oral cavity already established for each type of herbal product. Each herb has its concentration of specific efficacy and substantivity that must be explored. Another limitation regards the composition of the herbal product, which contained other ingredients that may have exerted a synergistic effect, thereby impeding the determination of the isolated effect of the herbal product. Moreover, few studies (Bhat, 2014; Baratakke et al., 2017; Grawish et al., 2016; Balappanavar et

al., 2013) reported the side effects of the herbs, which is an important aspect to consider for the safe clinical indication of these products.

Conclusion

The present systematic review demonstrated that the majority of herbal products used as an adjuvant to oral hygiene achieved better results in comparison to a placebo mouthrinse. The larger effect sizes found for *Camelia sinensis, Azadirachta indica, Anacardium occidentale* Linn, *Schinus terebinthifolius* and *Curcuma longa* make these herbs promising products for the control of dental plaque and gingival inflammation. However, the majority of studies had biases that compromise the degree of certainty in these conclusions. It is therefore necessary for further randomized clinical trials to be conducted with better methodological quality and the evaluation of possible adverse effects before these products can be recommended for use.

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References

Ainamo, J., Bay, I. (1975). Problems and proposals for recording gingivitis and plaque. *International Dental Journal***,** 25(4), 229–35.

American Dental Association. (2008) Acceptance Program Guidelines: Chemotherapeutic Products for Control of Gingivitis. Council on Scientific Affairs, Available at: http://www.ada.org/ en/science-research/ada-seal-of-acceptance/how-to-earn-the-ada-seal/ guidelines-for-product-acceptance.

Balappanavar, A. Y., Sardana, V., & Singh, M. (2013). Comparison of the effectiveness of 0.5% tea, 2% neem and 0.2% chlorhexidine mouthwashes on oral health: a randomized control trial. *Indian Journal of Dental Research : Official Publication of Indian Society for Dental Research*, *24*(1), 26–34. Comparative Study, Journal Article, Randomized Controlled Trial, Research Support, Non-U.S. Gov't. https://doi.org/10.4103/0970-9290.114933

Baratakke, S., Raju, R., Kadanakuppe, S., Savanur, N., Gubbihal, R., & Kousalaya, P. (2017). Efficacy of triphala extract and chlorhexidine mouth rinse against plaque accumulation and gingival inflammation among female undergraduates: A randomized controlled trial. *Indian Journal of Dental Research*, *28*(1), 49. https://doi.org/10.4103/0970-9290.203622

Barnett, M. L. (2006). The rationale for the daily use of an antimicrobial mouthrinse. *Journal of the American Dental Association (1939)*, *137 Suppl*, 16S–21S.

Journal Article, Review.
Bhat, N., Mitra, R., Oza, S., Mantu, V. K., Bishnoi, S., Gohil, M., & Gupta, R. (2014). The antiplaque effect of herbal mouthwash in comparison to chlorhexidine in human gingival disease: A randomized placebo controlled clinical trial. *Journal of Complementary and Integrative Medicine*, *11*(2), 129–137. https://doi.org/10.1515/jcim-2014-0002
Bhate, D., Jain, S., Kale, R., & Muglikar, S. (2015). The comparative effects of 0.12% chlorhexidine and herbal oral rinse on dental plaque-induced gingivitis: A randomized clinical trial. *Journal of Indian Society of Periodontology*, *19*(4), 393. https://doi.org/10.4103/0972-124X.153478
Boffetta, P., Hayes, R. B., Sartori, S., Lee, Y.-C. A., Muscat, J., Olshan, A., … Hashibe, M. (2016). Mouthwash use and cancer of the head and neck. *European Journal of Cancer Prevention*, *25*(4), 344–348. https://doi.org/10.1097/CEJ.0000000000000179
Cole, P., Rodu, B., & Mathisen, A. (2003). Alcohol-containing mouthwash and oropharyngeal cancer: a review of the epidemiology. *Journal of the American Dental Association (1939)*, *134*(8), 1079–87. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12956348
Dany, S. S., Mohanty, P., Tangade, P., Rajput, P., & Batra, M. (2015). Efficacy of 0.25% lemongrass oil mouthwash: A three arm prospective parallel clinical study. *Journal of Clinical and Diagnostic Research*, *9*(10), ZC13-ZC17. https://doi.org/10.7860/JCDR/2015/14465.6581
Drevon, D., Fursa, S. R., Malcolm, A. L. (2016) Intercoder Reliability and Validity of WebPlotDigitizer in Extracting Graphed Data. *Behav Modif*, 18.
Eisenberg, D. M., Davis, R. B., Ettner, S. L., Appel, S., Wilkey, S., Van Rompay, M., & Kessler, R. C. (1998). Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. *JAMA*, *280*(18), 1569–75. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9820257
Escribano, M., Figuero, E., Martin, C., Tobias, A., Serrano, J., Roldan, S., & Herrera, D. (2016). Efficacy of adjunctive anti-plaque chemical agents: a systematic review and network meta-analyses of the Turesky modification of the Quigley and Hein plaque index. *Journal of Clinical Periodontology*, *43*(12), 1059–1073. Journal Article, Meta-Analysis, Review. https://doi.org/10.1111/jcpe.12616
Gomes, C. E. B., Cavalcante, D. G., Filho, J. E. G., da Costa, F. N., & da Silva Pereira, S. L. (2016). Clinical effect of a mouthwash containing Anacardium occidentale Linn. on plaque and gingivitis control: A randomized controlled trial. *Indian Journal of Dental Research : Official Publication of Indian Society for Dental Research*, *27*(4), 364–369. Journal Article, Randomized Controlled Trial. https://doi.org/10.4103/0970-9290.191883
Grawish, M. E., Anees, M. M., Elsabaa, H. M., Abdel-Raziq, M. S., & Zedan, W. (2016). Short-term effects of Verbena officinalis Linn decoction on patients suffering from chronic generalized gingivitis: Double-blind randomized controlled multicenter clinical trial. *Quintessence International (Berlin, Germany : 1985)*, *47*(6), 491–498. Journal Article, Multicenter Study, Randomized Controlled Trial. https://doi.org/10.3290/j.qi.a35521
Grossman, E., Meckel, A. H., Isaacs, R. L., Ferretti, G. A., Sturzenberger, O. P., Bollmer, B. W., … Manhart, M. D. (1989). A clinical comparison of antibacterial mouthrinses: effects of chlorhexidine, phenolics, and sanguinarine on dental plaque and gingivitis. *Journal of Periodontology*, *60*(8), 435–440. Clinical Trial, Comparative Study, Journal Article, Research Support, Non-U.S. Gov't. https://doi.org/10.1902/jop.1989.60.8.435

Gunsolley, J. C. (2010). Clinical efficacy of antimicrobial mouthrinses. *Journal of Dentistry*, *38 Suppl 1*, S6-10. Journal Article, Review. https://doi.org/10.1016/S0300-5712(10)70004-X

Gupta, D., Bhaskar, D., Gupta, R., Karim, B., Jain, A., Singh, R., & Karim, W. (2014a). A randomized controlled clinical trial of Ocimum sanctum and chlorhexidine mouthwash on dental plaque and gingival inflammation. *Journal of Ayurveda and Integrative Medicine*, *5*(2), 109. https://doi.org/10.4103/0975-9476.131727

Gupta, D., Bhaskar, D. J., Gupta, R. K., Karim, B., Gupta, V., Punia, H., … Singh, P. (2014b). Effect of Terminalia chebula extract and chlorhexidine on salivary pH and periodontal health: 2 weeks randomized control trial. *Phytotherapy Research : PTR*, *28*(7), 992–998. Journal Article, Randomized Controlled Trial. https://doi.org/10.1002/ptr.5075

Gupta, D., Gupta, R. K., Bhaskar, D. J., & Gupta, V. (2015). Comparative evaluation of terminalia chebula extract mouthwash and chlorhexidine mouthwash on plaque and gingival inflammation - 4-week randomised control trial. *Oral Health & Preventive Dentistry*, *13*(1), 5–12. Comparative Study, Journal Article, Randomized Controlled Trial. https://doi.org/10.3290/j.ohpd.a32994

Gupta, D., & Jain, A. (2015). Effect of Cinnamon Extract and Chlorhexidine Gluconate (0.2%) on the Clinical Level of Dental Plaque and Gingival Health: A 4-Week, Triple-Blind Randomized Controlled Trial. *Journal of the International Academy of Periodontology*, *17*(3), 91–98. Comparative Study, Journal Article, Randomized Controlled Trial.

Haas, A. N., Wagner, T. P., Muniz, F. W. M. G., Fiorini, T., Cavagni, J., & Celeste, R. K. (2016). Essential oils-containing mouthwashes for gingivitis and plaque: Meta-analyses and meta-regression. *Journal of Dentistry*, *55*, 7–15. Review, Journal Article. https://doi.org/10.1016/j.jdent.2016.09.001

Higgins, J. P., & Green, S. (n.d.). Cochrane Handbook for Systematic Reviews of Interventions Cochrane Book Series THE COCHRANE COLLABORATION ®. Retrieved from https://pdfs.semanticscholar.org/4b43/91c08c45ebfcd046a53106c97ca09fcdf9fa.pdf

Hirasawa, M., Takada, K., Makimura, M., & Otake, S. (2002). Improvement of periodontal status by green tea catechin using a local delivery system: a clinical pilot study. *Journal of Periodontal Research*, *37*(6), 433–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12472837

Jayakumar, A., Naveen, A., Butchibabu, K., Reddy, P., Muralikrishna, T., & Chandrahas, B. (2012). A randomized, double-blind clinical study to assess the antiplaque and antigingivitis efficacy of Aloe vera mouth rinse. *Journal of Indian Society of Periodontology*, *16*(4), 543. https://doi.org/10.4103/0972-124X.106905

Katsura, H., Tsukiyama, R.I, Suzuki, A, Kobayashi, M. (2001). In Vitro Antimicrobial Activties of Bakuchiol agoinst oral microorganisms. *Antimicrobial Agents and Chemotherapy*, Washington,.45(11), 3009-3013.

Kothiwale, S., Patwardhan, V., Gandhi, M., Sohoni, R., & Kumar, A. (2014). A comparative study of antiplaque and antigingivitis effects of herbal mouthrinse containing tea tree oil, clove, and basil with commercially available essential oil mouthrinse. *Journal of Indian Society of Periodontology*, *18*(3), 316. https://doi.org/10.4103/0972-124X.134568

Kushiyama, M., Shimazaki, Y., Murakami, M., & Yamashita, Y. (2009). Relationship Between Intake of Green Tea and Periodontal Disease. *Journal of Periodontology*, *80*(3), 372–377. https://doi.org/10.1902/jop.2009.080510

Lauten, J. D., Boyd, L., Hanson, M. B., Lillie, D., Gullion, C., & Madden, T. E. (2005). A clinical study: Melaleuca, Manuka, Calendula and green tea mouth rinse. *Phytotherapy Research*, *19*(11), 951–957. https://doi.org/10.1002/ptr.1763

Li, X.C., Cai, L., Wu, C.D. (1997) Antimicrobial compounds from Ceanothus americanus against oral pathogens**.** *Phytochemistry*, 46(1), 97-102.

Lins, R., Vasconcelos, F. H. P., Leite, R. B., Coelho-Soares, R. S., & Barbosa, D. N. (2013). Avaliação clínica de bochechos com extratos de Aroeira (Schinus terebinthifolius) e Camomila (Matricaria recutita L.) sobre a placa bacteriana e a gengivite. *Revista Brasileira de Plantas Medicinais*, *15*(1), 112–120. https://doi.org/10.1590/S1516-05722013000100016

Lobene, R. R., Weatherford, T., Ross, N.M. (1986). Lamm, R.A., Menaker, L. A modified gingival index for use in clinical trials. *Clin Prev Dent*; 8, 3-6.

Löe, H., SIlness, J. (1963). Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand***,** 21, 533-551.

Löe, H., Theilade, E., & Jensen, S. B. (1965). EXPERIMENTAL GINGIVITIS IN MAN. *The Journal of Periodontology*, *36*, 177–187. Journal Article. https://doi.org/10.1902/jop.1965.36.3.177

Mackinnon, A. (2010). The use and reporting of multiple imputation in medical research – a review. *Journal of internal medicine*, *268*(6), 586-593

Mahyari, S., Mahyari, B., Emami, S. A., Malaekeh-Nikouei, B., Jahanbakhsh, S. P., Sahebkar, A., & Mohammadpour, A. H. (2016). Evaluation of the efficacy of a polyherbal mouthwash containing Zingiber officinale, Rosmarinus officinalis and Calendula officinalis extracts in patients with gingivitis: A randomized double-blind placebo-controlled trial. *Complementary Therapies in Clinical Practice*, *22*(2016), 93–98. https://doi.org/10.1016/j.ctcp.2015.12.001

Malhotra, R., Grover, V., Kapoo,r A., Saxena D. (2011). Comparison of the effectiveness of a commercially available herbal mouthrinse with chlorhexidine gluconate at the clinical and patient level. *Journal of Indian Society of Periodontology,* 15(4):349.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & PRISMA Group. (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Medicine*, *6*(7), e1000097. https://doi.org/10.1371/journal.pmed.1000097

Mühlemann H R & Son S.1971. Gingival sulcus bleeding. A leading symptom in initial gingivitis. *Helv Odont Acta*, 15, 107.

Nogales-Gaete J. (2005) Medicina alternativa y complementaria. *Rev Chil Neuro-Psiquiatr*,42(4):243-50.

Osso, D., & Kanani, N. (2013). Antiseptic mouth rinses: an update on comparative effectiveness, risks and recommendations. *Journal of Dental Hygiene : JDH*, *87*(1), 10–18. Comparative Study, Journal Article, Review.

Pereira, S. L. da S., de Oliveira, J. W. G., Angelo, K. K. S. V., da Costa, A. M. A., & Costa, F. (2011). Clinical effect of a mouth rinse containing Ocimum gratissimum on plaque and gingivitis control. *The Journal of Contemporary Dental Practice*, *12*(5), 350–355. Comparative Study, Journal Article, Randomized Controlled Trial.

Pradeep, A. R., Suke, D. K., Martande, S. S., Singh, S. P., Nagpal, K., & Naik, S. B. (2016). Triphala, a New Herbal Mouthwash for the Treatment of Gingivitis: A Randomized Controlled Clinical Trial. *Journal of Periodontology*, *87*(11), 1352–1359. https://doi.org/10.1902/jop.2016.130406

Priya, M., Anitha, V., Shanmugam, M., Ashwath, B., Sylva, S., & Vigneshwari, S. (2015). Efficacy of chlorhexidine and green tea mouthwashes in the

management of dental plaque-induced gingivitis: A comparative clinical study. *Contemporary Clinical Dentistry*, *6*(4), 505. https://doi.org/10.4103/0976-237X.169845

Quigley GA, Hein JW. (1962) Comparative cleansing efficiency of manual and power brushing. *J Am Dent Assoc*; 65:26-29.

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rassameemasmaung, S., Phusudsawang, P., & Sangalungkarn, V. (2013). Effect of green tea mouthwash on oral malodor. *ISRN Preventive Medicine*, *2013*, 975148. Journal Article. https://doi.org/10.5402/2013/975148

Sakanaka, S., Aizawa, M., Kim, M., & Yamamoto, T. (1996). Inhibitory effects of green tea polyphenols on growth and cellular adherence of an oral bacterium, Porphyromonas gingivalis. *Bioscience, Biotechnology, and Biochemistry*, *60*(5), 745–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8704303

Satpathy, A., Ravindra, S., Porwal, A., Das, A. C., Kumar, M., & Mukhopadhyay, I. (2013). Effect of alcohol consumption status and alcohol concentration on oral pain induced by alcohol-containing mouthwash. *Journal of Oral Science*, *55*(2), 99–105. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23748448

Saxer, U.P.; Muhlemann, H.R. (1975). `Motivation und Auftklarung", Schweizen Monatlischeschrift Zahnheilkunde, 85(9), 905-919.

Saxer, U. P., Stauble, A., Szabo, S. H., & Menghini, G. (2003). [Effect of mouthwashing with tea tree oil on plaque and inflammation]. *Schweizer Monatsschrift fur Zahnmedizin = Revue mensuelle suisse d'odonto-stomatologie = Rivista mensile svizzera di odontologia e stomatologia*, *113*(9), 985–996. Clinical Trial, English Abstract, Journal Article, Randomized Controlled Trial.

Serrano, J., Escribano, M., Roldan, S., Martin, C., & Herrera, D. (2015). Efficacy of adjunctive anti-plaque chemical agents in managing gingivitis: a systematic review and meta-analysis. *Journal of Clinical Periodontology*, *42 Suppl 1*, S106-38. Journal Article, Meta-Analysis, Research Support, Non-U.S. Gov't, Review. https://doi.org/10.1111/jcpe.12331

Silness J, Löe H. (1964). Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition*. Acta Odontol Scand*, 22:121-135.

Silva, M. I. G., Melo, C. T. V. de, Vasconcelos, L. F., Carvalho, A. M. R. de, & Sousa, F. C. F. (2012). Bioactivity and potential therapeutic benefits of some medicinal plants from the Caatinga (semi-arid) vegetation of Northeast Brazil: a review of the literature. *Revista Brasileira de Farmacognosia*, *22*(1), 193–207. https://doi.org/10.1590/S0102-695X2011005000171

Shaw, L., Murray, J.J. (1977). A new index for measuring extrinsic stain in clinical trials. *Community Dent Oral Epidemiol*, 5:116-120.

Stoeken, J. E., Paraskevas, S., & van der Weijden, G. a. (2007). The long-term effect of a mouthrinse containing essential oils on dental plaque and gingivitis: a systematic review. *Journal of Periodontology*, *78*(7), 1218–28. https://doi.org/10.1902/jop.2007.060269

Van der Weijden, G. A., & Hioe, K. P. K. (2005). A systematic review of the effectiveness of self-performed mechanical plaque removal in adults with gingivitis using a manual toothbrush. *Journal of Clinical Periodontology*, *32 Suppl 6*, 214–228. Journal Article, Meta-Analysis, Review. https://doi.org/10.1111/j.1600-051X.2005.00795.x

Van Strydonck, D. A. C., Slot, D. E., Van Der Velden, U., & Van Der Weijden, F. (2012). Effect of a chlorhexidine mouthrinse on plaque, gingival inflammation

and staining in gingivitis patients: A systematic review. *Journal of Clinical Periodontology*, *39*(11), 1042–1055. https://doi.org/10.1111/j.1600-051X.2012.01883.x

Varoni, E. M., Lodi, G., Sardella, A., Carrassi, A., & Iriti, M. (2012). Plant polyphenols and oral health: old phytochemicals for new fields. *Current Medicinal Chemistry*, *19*(11), 1706–1720. Journal Article, Review.

Waghmare, P. F., Chaudhari, A. U., Karhadkar, V. M., & Jamkhande, A. S. (2011). Comparative evaluation of turmeric and chlorhexidine gluconate mouthwash in prevention of plaque formation and gingivitis: A clinical and microbiological study. *Journal of Contemporary Dental Practice*, *12*(4), 221–224. https://doi.org/10.5005/jp-journals-10024-1038

World Health Organization (1977) – WHO. WHA 30.49 Promotion and development of training and research in traditional medicine**.** In: 30th. World Healtyh Assembly; 2-19,Geneva..

World Health Organization (1978) - WHO. Drug policies and management: Medicinal Plants. WHO Document WHA 31.33. Geneva.

Zamora-Perez, A. L., Mariaud-Schmidt, R. P., Fuentes-Lerma, M. G., Guerrero-Velázquez, C., Gómez-Meda, B. C., López-Verdín, S., & Zúñiga-González, G. M. (2013). Increased number of micronuclei and nuclear anomalies in buccal mucosa cells from people exposed to alcohol-containing mouthwash. *Drug and Chemical Toxicology*, *36*(2), 255–260. https://doi.org/10.3109/01480545.2012.737804

Table 1. Search strategy Pubmed/ MEDLINE

Search	Items found
#1 (intervention)	
"Drugs, Chinese Herbal"[Mesh] OR “Chinese Drugs, Plant” OR “Chinese Herbal Drugs” OR “Herbal Drugs, Chinese” OR “Plant Extracts, Chinese” OR “Chinese Plant Extracts” OR “Extracts, Chinese Plant” OR "Herbal Medicine"[Mesh] OR “Medicine, Herbal” OR “Herbalism” OR "Phytotherapy"[Mesh] OR "Phytotherapy" OR “Herbal Therapy” OR “Herb Therapy” OR "Plants, Medicinal"[Mesh] OR "Plant Extracts"[Mesh] OR “Herbal*” AND “Mouthwashes” [Mesh] OR “Mouthwashe*” OR “Mouthrinse” OR “Rinse*” OR “Oral Rinse”	17984
#2 (outcome)	
"Gingivitis"[Mesh] OR “Gingivitis” OR “Gingivitides” OR "Gingival Pocket"[Mesh] OR “Pocket, Gingival” OR “Gingival Pockets” OR “Pockets, Gingival” OR “Gingival*” OR “Index, Gingival” OR “Indices, Gingival” OR “Dental Plaque”[Mesh] OR “Plaque, Dental” OR “Dental Plaque Index”[Mesh] OR “Index, Dental Plaque” OR “Dental Plaque Indexes” OR “Indexes, Dental Plaque” OR “Dental Plaque Indices” OR “Indices, Dental Plaque” OR “Biofilms”[Mesh] OR “Biofilm*”	95637
#2 (type of study)	
((clinical[Title/Abstract] AND trial[Title/Abstract]) OR clinical trials as topic[MeSH Terms] OR clinical trial[Publication Type] OR random*[Title/Abstract] OR random allocation[MeSH Terms] OR therapeutic use[MeSH Subheading])	4883400
((#1) AND #3) AND #2	2.690

Table 2. Characteristics of selected studies

Reference Year Country	Design/ Duration	Intervention	Number of subjects	Rinsing regimen	Gender Mean age ± SD	Index
Baratakke et al. (2017) India	Parallel 21 days	**A:** 0.6% *Emblica officinalis* (Amalaki), *Termin alia chebula* (Haritaki), and *Terminalia belerica* (Bibhitaki) - **Triphala®** **B**: 0.12% CHX - **Hexidine®** **C**: Placebo	**A**:20 **B**:20 **C**:20	2 x 10 mL x 30'' BP: (?) Without any oral hygiene supervision	**Gender** 60♀ **Mean age** **A**: 21 (?) **B**: 21 (?) **C:** 21 (?)	PI (S & L) GI (S & L)
Gomes et al. (2016) Brazil	Crossover 1 month	**A:** 10% *Anacardium occidentale* Linn - (?) **B:** 0.12% CHX - (?) **C:** Placebo	**A:** 10 **B**: 10 **C:** 10	2 x (?) x 60' BP: (?) Without any oral hygiene supervision	**Gender** 15♂15 ♀ **Mean age** **A:** 25(?) **B:** 25(?) **C:** 25(?)	PII (Q & H) GBI (A & B)
Grawish et al. (2016) Egypt	Parallel 28 days	**A:** 240 mL *Verbena officinalis Linn* - (?) **C:** Placebo	**A:** 130 **B:** 130	2 x 10 mL x 60'' BP: (?) Supervised oral hygiene	**Gender** **A:**95♂34 ♀ **B:** 88♂42 ♀ **Mean age** **A:**45.67 ± 4.56 **B:**45.7 ± 4.6	PII (Q & H) GI (S & L)
Mahyari et al. (2016) Iran	Parallel 14 days	**A:** 5% *Zingiber officinale*, *Rosmarinus officinalis and Calendula officinalis* (?) **B**: 0.2% CHX (?) **C:** Placebo	**A:** 20 **B:** 20 **C:** 20	2 x10 mL x 30" BP: (?) (?)	**Gender** **A:** 8 ♂12 ♀ **B:** 8 ♂12 ♀ **C:** 9 ♂11 ♀ **Mean age** **A:** 36.2 ± 11.65 **B:** 35.9 ± 11.93 **C:** 35 ± 11.52	PI (Q & H) MGI (Lobene, 1986)
Pradeep et al.(2016) India	Parallel 2 months	**A:** 6% *Emblica officinalis*, *Terminalia chebula* and *Terminalia belerica* - **Triphala ®** **B:** 0.2% CHX - (?) **C:** Placebo	**A:** 30 **B:** 30 **C:** 30	2 x 15ml x (?) BP: Yes (?)	**Gender** **A:** 13♂; 17 ♀ **B:** 15♂; 15 ♀ **C :** 14♂; 16 ♀ **Mean age** **A:**30.17 ± 6.1 **B:**29.90 ±5.6 **C:**29.70 ± 6.2	PI (Q & H) GI (S & L)
Bhate et al. (2015) India	Parallel 21 days	**A:** (?) Piper betle (Nagavalli), Bhibhitika (*Terminalia bellerica*), Pilu (*Salvadora persica*) - **Hiora®** **B:** 0.12% CHX - **Peridex®**	**A:** 76 **B:** 76	2 x 15ml x 30'' BP: (?) Supervised oral hygiene	**Gender** (?) **Mean age** **A:** 35 (?) **B:** 35 (?)	PI (S & L) GI (S & L)
Dany et al. (2015) India	Parallel 21days	**A:** 0.25% *Cymbopogan citratus* - (?) **B:** 0.2% CHX - (?) **C:** Oral prophylaxis only	**A:** 20 **B:** 20 **C:** 20	2 x 60'' x (?) BP: yes (?)	**Gender** (?) **Mean age** **A:** 35 (?) **B:** 35 (?) **C:** 35(?)	PI (S & L) GI (S & L)
Gupta & Jain (2015) India	Parallel 1 month	**A:** 20%*Cinnamomum zeylanicum*- (?) **B:** 0.2% CHX - (?) **C:** Placebo	**A:** 35 **B:** 35 **C:** 35	2 x 10 mL x 60'' BP: yes Supervised oral hygiene	**Gender** 17♂; 18 ♀ 16 ♂; 19 ♀ 20 ♂; 15 ♀ **Mean age** **A:** 23.17 ±2.45 **B:** 22.72 ± 2.08 **C:** 23.13 ± 2.39	PI (Q & H) GI (S & L)

Gupta et al (2015) India	Parallel 1 month	**A:** 10% *Terminalia chebula* (Haritaki)-(?) **B**: 0.12% CHX - (?) **C:** Placebo	**A:** 30 **B:** 30 **C:** 30	2 x10 mL x 60” BP: no (?)	**Gender** (?) **Mean age** **A:** 22.16(?) **B:** 21.71(?) **C:** 22.13(?)	PI (Q & H) GI (S & L)
Priya et al. (2015) India	Parallel 1 month	**A*:*** 5% *Camellia sinensis*-(?) **B:** 0.12% CHX - (?) .	**A:** 15 **B:** 15	30 min after oral hygiene BP: no Supervised oral hygiene	**Gender** (?) **Mean age** **A:** 21(?) **B:** 21(?)	PI (Q & H) GI (S & L)
Bhat et al. (2014) India	Parallel 1 month	**A:** (?) Pilu (*S. persica*), Bibhitaka (*T. bellerica*), Nagavalli (*P. betle*), Gandhapura taila (*G. fragrantissima*), cardamom (*E. cardamomum*), mint (*Mentha spp*.) and Yavani satva (*Trachyspermum ammi*) - (**Hiora®**) **B:** 0.2% CHX - **ClohexPlus®** **C:** Placebo	**A:** 24 **B:** 24 **C:** 24	2x 10 Ml x 60” BP: (?) (?)	**Gender** 37♂; 35 ♀ **Mean age** **A:** 20.2 ± 0.7 **B:** 20.95 ± 0.1 **C:** 20.79 ± 1.2	PI (Q & H) GI (S & L)
Gupta et al. (2014a) India	Parallel 1 month	**A*:*** 4% *Ocimum gratissimum*-(?) **B:** 0.12% CHX (?) **C:** Placebo	**A:** 36 **B:** 36 **C:** 36	2 x 10 mL x 60” BP: no (?)	**Gender** (?) **Mean age** **A:** 23.16(?) **B:** 22.71(?) **C:** 24.13(?)	PI (Q & H) GI (S & L)
Gupta et al. (2014b) India	Parallel 14days	**A:** 10%*Terminalia chebula* (Haritaki) -(?) **B:** 0.12% CHX - (?) **C:** Placebo	**A:** 26 **B:** 26 **C:** 26	2x 10ml x 60’’ BP: no (?)	**Gender** 39♂; 39♀ **Mean age** **A:** 22.20(?) **B:** 21.44(?) **C:** 22.11(?)	PI (Q & H) GI (S & L)
Kothiwale et al. (2014) India	Parallel 21 days	**A:** 25%*Melaleuca alternifolia, Syzygium aromaticum* and *Ocium basilicum* - (?) **D:** EO (?)	**A:** 25 **B:** 25	2 x10 mL x 30” BP: yes Supervised oral hygiene	**Gender** (?) **Mean age** **A:** 30.05 ± 0.11 **B:** 37.2 ± 13.63	PI (S & L) GI (S & L)
Balappanavar et al. (2013) India	Parallel 21 days	**A:** 0.5% *Camellia sinensis* - (?) **A:** 2%*Azadirachta indica* - (?)(Neem) **B:** 0.2% CHX - **Periogard®**	**A:** 10 **B:** 10 **C:** 10	2 x 15 mL x 30” BP: (?) Supervised oral hygiene	**Gender** **A:** 5 ♂; 5 ♀ **B:**5 ♂; 5 ♀ **C:**5 ♂; 5 ♀ **Mean age** **A:** 21.2 ± 1.86 **B:** 20.9 ± 0.78 **C:** 20.7 ± 1.87	PI (S & L) GI (S& L)
Lins et al. (2013) Brazil	Parallel 15 days	**A:** (?) *Matricaria chamomilla Schinus terebinthifolius* - (?) **A:** *(?) Schinus terebinthifolius* - (?) **B**: 0.12% CHX (?)	**A:** 20 **B:** 20 **C:** 19	2 x (?) x 60” BP: yes Supervised oral hygiene	**Gender** 20 ♂ 39♀ **Mean age** **A:** 34 (?) **B:** 34 (?) **C:** 34 (?)	PI (S & L) GBI (A & B) .

Rassameemasmaung et al. (2013) Thailand	Parallel 28 days	**A:**(?) *Camellia sinensis* - (?) **C:** Placebo	**A:** 30 **B:** 30	2 x 15mL x 60'' BP: (?) Supervised oral hygiene	**Gender** **A:** 3 ♂; 27 ♀ **B:** 3 ♂; 27 ♀ **Mean age** **A:** 27.2 ± 9.9 **B:** 25.8 ± 7.6	PI (S & L) PBI (S & M)
Jayakumar et al. (2012) India	Parallel 7days	**A:** 100% *Aloe vera*- (?) **B:** 0.2% CHX - (?) **C:** Placebo	**A:** 40 **B:** 40 **C:** 40	2 x 10 mL x 60" BP: yes Without any oral hygiene supervision	**Gender** 58♂; 62♀ **Mean age** **A:** 21(?) **B:** 21(?) **C:** 21(?)	PI (Q & H) MGI (Lobene,1986)
Pereira et al. (2011) Brazil	Parallel 3 months	**A:** (?) *Ocimum gratissimum*-(?) **B**: 0.12% CHX - (?) ***C**:* Placebo	**A:** 10 **B:** 10 **C:** 10	3 x 10 mL x 60" BP: yes Supervised oral hygiene	**Gender** 15♂; 15 ♀ **Mean age** **A:** 35(?) **B**: 35(?) **C:** 35(?)	PI (Q & H) GBI (A & B)
Waghmare et al. (2011) India	Parallel 21days	**A:** 10 mg *Curcuma longa* and 0.005% *Mentha spp*.-(?) **B:** 0.2% CHX- (?)	**A:** 50 **B:** 50	2 x 10 ml x (?) BP: (?) Supervised oral hygiene	**Gender** (?) **Mean age** **A:** 30 (?) **B:** 30 (?)	PI (Q & H) GI (S & L)
Lauten et al. (2005) USA	Parallel 3 months	**A:** 0.67% Melaleuca *alternifolia,* 0.33%,*Leptospermum scoparium*(Manuka), 1% Calendula flower(Calendula), 0.5% *Camellia sinensis* (green tea)- (?) **C:** Placebo	**A:** 8 **B:** 9	2 x 15 mL x 30" BP: (?) (?)	**Gender** **A:** 1♂; 7 ♀ **B:** 2 ♂; 7 ♀ **Mean age** A: 31.88 ± 7.51 B: 47.33 ± 15.50	PI (Q & H) GI (S & L)
Saxer et al. (2003) Germany	Parallel 3 months	**A:**1.5% *Melaleuca alternifolia* and 10% xylitol - **Tebodont®** **C:** Placebo	**A:** 13 **B:** 13	3 x 10 mL x 90" BP: (?) (?)	**Gender** (?) **Mean age** **A:** 35(?) **B**: 35(?) **C:** 35(?)	PI (Q & H) GI (M &S)
Grossman et al. (1989) USA	Parallel 6 months	**A:** 0.03%*Sanguinarine extract and zinc* - **Veadent®** **B:**0.12% CHX **Peridex®** **C:** Placebo **D:** OE - **Listerine®**	**A:** 113 **B:** 129 **C:** 127 **D:** 133	**A:** 15mL x 15" **B:** 20mL x 30" **C:** 15mL x 30'' **D:** 15mL x 30'' BP: yes (?)	**Gender** **A:** 42♂; 85 ♀ **B:** 46 ♂; 83♀ **C:**35♂; 78 ♀ **D:** 43 ♂; 30 ♀ **Mean age** **A:**35.9 **B:**37.1 **C:**36.8 **D:** 36.5	PI (Q & H) GI (S & L)

BP, baseline prophylaxis; (?), not reported; ♂ male; ♀ female; GI (S & L), PII (S & L), PI (Q & H), GBI (A & B), PBI (S & M), GI (M &S), PI (S & M); MGI (LOBENE,1986) A, herbal rinse; B, control rinse; C, placebo rinse; D, OE (essential oils) rinse

Table 3. Characteristics of means and standard deviations for dental plaque and gingivitis parameters.

Study	**Dental plaque** Base Score Mean ± SD	**Dental plaque** End Score Mean ± SD	**Gingival inflammation** Base Score Mean ± SD	**Gingival inflammation** End Score Mean ± SD	**∆SD (%)**	**Authors' conclusions**
Baratakke et al. (2017)	**A:** 1.13 ± 0.21 **B:** 1.22 ± 0.18 **C:** 1.22 ± 0.19	**A:** 0.90 ± 0.24 **B:** 0.92 ± 0.26 **C:** 1.27 ± 0.24	**A:** 1.24 ± 0.27 **B:** 1.23 ± 0.29 **C:** 1.23 ± 0.18	**A:** 0.90 ± 0.32 **B:** 0.94 ± 0.37 **C:** 1.27 ± 0.16	**Plaque** **A=** -0.23 ± 0.31 (-20.3%)† **B=** 0.3 ± 0.31 (-24.5%)† **C=** 0.05 ± 0.30 (-4.0%) **Gingivitis** **A=** -0.34 ± 0.41 (-27.4%)† **B=** -0.29 ± 0.47 (-23.5%)† **C=** 0.04 ± 0.24 (-3.2%)	No significant difference between Triphala and CHX for dental plaque or gingivitis; both products significantly better than placebo solution ($p < 0.001$).
Gomes et al. (2016)	**A:** 0.71 ± 0.19 **B:** 0.67 ± 0.18 **C:** 0.70 ± 0.13	**A:** 0.49 ± 0.21 **B:** 0.47 ± 0.16 **C:** 0.66 ± 0.03	**A:** 0.30 ± 0.08 **B:** 0.34 ± 0.10 **C:** 0.20 ± 0.04	**A:** 0.13 ± 0.10 **B:** 0.15 ± 0.09 **C:** 0.14 ± 0.08	**Plaque** **A=** -0.22 ± 0.28 (-30.98%)† **B=** -0.2 ± 0.24 (-29.85%)† **C=** -0.04 ± 0.13 (-5.71%) **Gingivitis** **A= -**0.17 ± 0.12 (-56.6%)† **B= -**0.19 ± 0.13 (-55.8%)† **C= -**0.06 ± 0.08 (-30%)	No significant difference between cashew and CHX for plaque or gingivitis; both products significantly better than placebo solution ($p > 0.05$).
Grawish et al. (2016) Egypt	**A:** 2.99 ± 0.35* **C:** 2.65 ± 0.35*	**A:** 1.07 ± 0.35* **C:** 1.19 ± 0.35*	**A:** 2.99 ± 0.35* **C:** 2.54 ± 0.35*	**A:** 1.31 ± 0.35* **C:** 1.35 ± 0.35*	**Plaque** **A=** -1.92 ± 0.49 (-64.2%)† **C=** -1.46 ± 0.49 (-55%) **Gingivitis** **A=** -1.68 ± 0.49 (-56.1%)† **C=** -1.19 ± 0.49 (-46.85%)	*Verbena officinalis Linn* significantly better than placebo solution for both dental plaque and gingivitis ($p < 0.001$).
Mahyari et al. (2016) Iran	**A:** 2.5 ± 0.35* **B:** 2 ± 0.35* **C:** 1 ± 0.35*	**A:** 2.5 ± 0.35* **B:** 2 ± 0.35* **C:** 1 ± 0.35*	**A:** 3 ± 0.35* **B:** 2.5 ± 0.35* **C:** 2 ± 0.35*	**A:** 0 ± 0.35* **B:** 0 ± 0.35* **C:** 2 ± 0.35*	**Plaque** ¶ **Gingivitis** ¶	No significant difference between herbal products tested (ginger, rosemary and *Calendula*) and CHX for dental plaque or gingivitis; all products significantly better than placebo solution ($p > 0.05$).
Pradeep et al. (2016) India	**A:** 4.39 ± 0.19 **B:** 4.57 ± 0.14 **C:** 4.49 ± 0.44	**A:** 1.90 ± 0.21 **B:** 1.84 ± 0.17 **C:** 2.82 ± 0.29	**A:** 2.69 ± 0.15 **B:** 2.67 ± 0.14 **C:** 2.68 ± 0.15	**A:** 0.86 ± 0.22 **B:** 0.80 ± 0.18 **C:** 1.76 ± 0.12	**Plaque** A= -2.49 ± 0.28 (-56.7%)† B= -2.73 ± 0.22 (-59.7%)† C= -1.67 ± 0.52 (-37.1%) **Gingivitis** A= -1.83 ± 0.26 (-68%)† B= -1.87 ± 0.22 (-70%)† C= -0.92 ± 0.19(-34.3%)	No significant difference between Triphala and CHX for plaque or gingivitis; both products significantly better than placebo solution.
Bhate et al. (2015) India	**A:** 1.76 ± 0.4 **B:** 1.76 ± 0.6	**A:** 1.01 ± 0.14 **B:** 0.15 ± 0.07	**A:** 1.90 ± 0.4 **B:** 1.92 ± 0.6	**A:** 1.10 ± 0.19 **B:** 0.15 ± 0.07	**Plaque** A= -0.75 ± 0.42 (-42.6%) B= -1.61 ± 0.60 (-91.4%)‡ **Gingivitis** A= -0.80 ± 0.44 (-42.1%) B= -1.77 ± 0.60 (-92.1%)‡	CHX significantly better than Hiora ($p < 0.001$) for dental plaque and gingivitis.

Dany et al. (2015) India	**A:** 1.85 ± 0.27 **B:** 1.87 ± 0.30 **C:** 1.90 ± 0.30	**A:** 1.20 ± 0.19 **B:** 1.21 ± 0.28 **C:** 1.43 ± 0.36	**A:** 2.19 ± 0.19 **B:** 2.23 ± 0.31 **C:** 2.18 ± 0.32	**A:** 1.59 ± 0.37 **B:** 1.67 ± 0.46 **C:** 1.99 ± 0.42	**Plaque** A= -0.65 ± 0.33 (-35.1%)† B= -0.66 ± 0.41 (-35.2%)† C= -0.47 ± 0.46 (-24.7%) **Gingivitis** A= -0.6 ± 0.41 (-27.3%)† B= -0.56 ± 0.55 (-25.1%)† C= -0.19 ± 0.52 (-8.7%)	No significant difference between lemon grass CHX for dental plaque or gingivitis; both products significantly better than placebo solution.
Gupta & Jain (2015) India	**A:** 2.4 ± 0.06 **B:** 2.5 ± 0.06 **C:** 2.5 ± 0.07	**A:** 1.2 ± 0.46 **B:** 1.0 ± 0.48 **C:** 2.9 ± 0.50	**A:** 2.7 ± 0.08 **B:** 2.9 ± 0.07 **C:** 2.5 ± 0.08	**A:** 1.1 ± 0.57 **B:** 0.7 ± 0.59 **C:** 2.8 ± 0.55	**Plaque** A= -1.2 ± 0.46 (-50%)† B= - 1.5 ± 0.48 (-60%)† C= 0.4 ± 0.50 (-16%) **Gingivitis** A= - 1.6 ± 0.57 (-59.2%)† B= -2.2 ± 0.59 (-75.8%)† C= 0.3 ± 0.55 (-12%)	No significant difference between cinnamon and CHX for dental plaque or gingivitis; both products significantly better than placebo solution.
Gupta et al. (2015) India	**A:** 2.89 ± 0.54 **B:** 3.00 ± 0.44 **C:** 2.98 ± 0.58	**A:** 2.59 ± 0.46 **B:** 2.38 ± 0.57 **C:** 3.22 ± 0.56	**A:** 2.33 ± 0.43 **B:** 2.36 ± 0.45 **C:** 2.38 ± 0.49	**A:** 1.45 ± 0.36 **B:** 1.31 ± 0.40 **C:** 2.42 ± 0.49	**Plaque** A= - 0.3 ± 0.70 (-10.3%)† B= -0.6 ± 0.72 (-20%)† C= 0.24 ± 0.80 (-8%) **Gingivitis** A= -0.88 ± 0.56 (-37.7%)† B= -1.05 ± 0.60 (-44.4%)† C= 0.04 ± 0.69 (-1.6%)	No significant difference between *Terminalia chebula* and CHX for dental plaque or gingivitis; both products significantly better than placebo solution.
Priya et al. (2015) India	**A:** 2.2 ± 0.4 **B:** 2.19 ± 0.3	**A:** 1.2 ± 0.3 **B:** 1.3 ± 0.2	**A:** 2.01 ± 0.4 **B:** 2.06 ± 0.1	**A:** 1.23 ± 0.2 **B:** 1.2 ± 0.2	**Plaque** A= -1 ± 0.5 (-45.4%) B= -0.89 ± 0.36 (40.6%) **Gingivitis** A= -0.78 ± 0.44 (-38.8%)+ B= -0.86 ± 0.22 (-41.7%)	Significant difference favoring green tea over CHX for gingivitis ($p < 0.05$). .
Bhat (2014) India	**A:** 1.5 ± 0.32 **B:** 1.22 ± 0.25 **C:** 1.34 ± 0.48	**A:** 0.78 ± 0.84* **B:** 0.68 ± 0.14 **C:** 1.46 ± 0.19	**A:** 0.62 ± 0.52 **B:** 0.77 ± 0.43 **C:** 0.91 ± 0.84	**A:** 0.35 ± 0.46 **B:** 0.42 ± 0.84* **C:** 1.02 ± 0.79	**Plaque** A= - 0.72 ± 0.89 (-48%)† B= -0.54 ± 0.28 (44.2%)† C= 0.12 ± 0.51 (-8.9%) **Gingivitis** A= -0.27 ± 0.69 (-43.5%)† B= -0.35 ± 0.94 (-56.4%)† C= 0.11 ± 1.15 (-12%)	No significant difference between HIORA and CHX for dental plaque or gingivitis; both products significantly better than placebo solution ($p< 0.004$).
Gupta et al. (2014a) India	**A:** 3.00 ± 0.44 **B:** 2.61 ± 0.54 **C:** 2.17 ± 0.68	**A:** 2.49 ± 0.46 **B:** 2.10 ± 0.57 **C:** 2.23 ± 0.56	**A:** 2.23 ± 0.43 **B:** 2.36 ± 0.45 **C:** 2.40 ± 0.49	**A:** 1.35 ± 0.36 **B:** 1.44 ± 0.40 **C:** 2.20 ± 0.49	**Plaque** A= -0.51 ± 0.63 (-17%) B= -0.51 ± 0.78 (-19.5%) C= 0.06 ± 0.88 (-2.7%) **Gingivitis** A= 0.88 ± 0.56 (-39.4%)	No significant difference between basil and CHX for dental plaque and gingivitis; CHX significantly better than placebo solution.

					B= -0.92 ± 0.60 (-38.9%) C= -0.2 ± 0.69 (-8.3%)	
Gupta et al. (2014b) India	**A:** 2.97 ± 0.44 **B:** 2.78 ± 0.54 **C:** 2.30 ± 0.58	**A**: 3.18 ± 0.58* **B:** 2.80 ± 0.58* **C:** 2.74 ± 0.58*	**A:** 2.0 ± 0.43 **B:** 2.40 ± 0.45 **C:** 2.45 ± 0.49	**A:** 1.88 ± 0.49* **B:** 2.01 ± 0.49* **C:** 2.60 ± 0.49*	**Plaque** A= 0.21 ± 0.72 (-7%) B= 0.02 ± 0.79 (-0.71%) C= 0.44 ± 0.82 (19.1%) **Gingivitis** A= -0.12 ± 0.65 (-6%) B= -0.39 ± 0.66 (-16.2%) C= 0.15 ± 0.69 (-6.1%)	No significant difference between *Terminalia chebula* and CHX for dental plaque or gingivitis; both products significantly better than placebo solution
Kothiwale et al. (2014) India	**A:** 1.63 ± 0.24 **D:** 1.81 ± 0.44	**A:** 0.59 ± 0.48 **D:** 0.51 ± 0.38	**A:** 1.59 ± 0.32 **D:** 1.76 ± 0.27	**A:** 0.38 ± 0.28 **D:** 0.53 ± 0.38	**Plaque** A= -1.04 ± 0.53 (-63.8%) D= -1.3 ± 0.58 (-71.8%) **Gingivitis** A= -1.21 ± 0.42 (-76.1%) D= -1.23 ± 0.46 (-69.8%)	No significant difference between herbal products and EO for dental plaque and gingivitis.
Balappanavar et al. (2013) India	**A:** 1.55 ± 0.03 **A:** 1.52 ± 0.06 **B:** 1.52 ± 0.06	**A:** 0.22 ± 0.44 **A:** 0.56 ± 0.53 **B:** 0.80 ± 0.46	**A:** 2.44 ± 0.73 **A:** 2.50 ± 0.71 **B:** 2.67 ± 1.00	**A:** 1.11 ± 0.6 **A:** 1.11 ± 0.6 **B:** 1.22 ± 0.83	**Plaque** A= -1.33 ± 0.44 (-85%) A= -0.96 ± 0.53 (-63.1%) B= -0.72 ± 0.46 (-47%)$^{+}$ **Gingivitis** A= -1.33 ± 0.94 (-54.5%) A= -1.39 ± 0.92 (-55.6%)$^{+}$ B= -1.45 ± 1.29 (-54.3%)$^{+}$	Green tea and Neem significantly better than CHX for gingivitis ($p < 0.05$).
Lins et al. (2013) Brazil	**A:** 1.34 ± 0.32 **A:** 0.99 ± 0.28 **B:** 1.23 ± 0.31	**A:** 0.30 ± 0.15 **A:** 0.28 ± 0.15 **B:** 0.26 ± 0.10	**A:** 0.44 ± 0.11 **A:** 0.41 ± 0.14 **B:** 0.49 ± 0.09	**A:** 0.18 ± 0.06 **B:** 0.15 ± 0.07 **B:** 0.18 ± 0.06	**Plaque** A= -1.04 ± 0.35 (-77.6%) A= -0.71 ± 0.31 (-71.7%) C= -0.97 ± 0.32 (-78.8%) **Gingivitis** A= -0.26 ± 0.12 (-59%) B= -0.26 ± 0.15 (-63.4%) C= 0.31 ± 0.10 (-63.2%)	No significant difference between chamomile and Brazilian pepper tree and CHX for dental plaque or gingivitis.
Rassameemasmaung et al. (2013) Thailand	**A:** 1.29 ± 0.30 **C:** 1.17 ± 0.27	**A:** 0.97 ± 0.24 **C:** 1.02 ± 0.25	**A:** 0.85 ± 0.24 **C:** 0.82 ± 0.35	**A:** 0.76 ± 0.25 **C:** 0.73 ± 0.29	**Plaque** A= -0.32 ± 0.38 (-29.8) C= -0.15 ± 0.36 (-12.8) **Gingivitis** A= -0.09 ± 0.34 (-10.5%)$^{+}$ C= -0.09 ± 0.45 (-10.9%)	Green tea significantly better than placebo solution for gingivitis.
Jayakumar et al. (2012) India	**A:** 1.51 ± 0.35* **B:** 1.56 ± 0.35* **C:** 1.66 ± 0.35*	**A:** 2.40 ± 0.35* **B:** 2.70 ± 0.35* **C:** 2.11 ± 0.35*	**A:** 0.07 ± 0.35* **B:** 0.45 ± 0.35* **C:** 0.15 ± 0.35*	**A:** 0.25 ± 0.35* **B:** 0.34 ± 0.35* **C:** 0.24 ± 0.35*	**Plaque** A: 0.89 ± 0.49 (-58.9%)† B: 1.14 ± 0.49 (-73%)†‡ C: 0.45 ± 0.49 (-27.1%) **Gingivitis** A: 0.18 ± 0.49 (-25%)† B: -0.11 ± 0.49 (-24.4%)	No significant difference between *Aloe vera* and CHX for dental plaque or gingivitis; both products significantly better than placebo solution.

					C: 0.09 ± 0.49 (6%)	
Pereira et al. (2011) Brazil	**A:** 1.08 ± 0.56 **B:** 1.55 ± 0.79 **C:** 1.59 ± 0.79	**A:** 0.62 ± 0.26 **B:** 0.55 ± 0.29 **C:** 1.69 ± 0.83	**A:** 0.22 ± 0.04 **B:** 0.27 ± 0.02 **C:** 0.23 ± 0.07	**A:** 0.08 ± 0.02 **B:** 0.06 ± 0.03 **C:** 0.24 ± 0.02	**Plaque** A= -0.46 ± 0.61 (-45.5%) B= -1 ± 0.84 (-64.1%) C= 0.1 ± 1.14 (-6.2%) **Gingivitis** A= 0.14 ± 0.44 (-63.6%) B= -0.21 ± 0.03 (-77.7%) C= 0.01 ± 0.07 (-43.47)	Significant difference favoring CHX over basil for dental plaque and gingivitis ($p < 0.05$); both products significantly better than placebo solution.
Waghmare et al. (2011) India	**A:** 3.27 ± 0.47 **B:** 3.31 ± 0.36	**A:** 1.22 ± 0.13 **B:** 0.83 ± 0.27	**A:** 1.81 ± 0.13 **B:** 1.77 ± 0.19	**A:** 0.71 ± 0.12 **B:** 0.73 ± 0.52	**Plaque** A= -2.05 ± 0.48 (-62.6%) B= -2.48 ± 0.45 (-74.9%)‡ **Gingivitis** A= -1.1 ± 0.17 (-60.7%) B= -1.04 ± 0.55 (-58.7%)	Significantly greater reduction in dental plaque with CHX compared to turmeric ($p < 0.05$); no difference between products regarding gingival in index.
Lauten (2005) USA	**A:** 1.21 ± 0.35 **C:** 1.52 ± 0.36	**A:** 1.39 ± 0.47 **C:** 1.62 ± 0.39	**A:** 1.00 ± 0.20 **C:** 0.96 ± 0.23	**A:** 0.94 ± 0.33 **C:** 0.88 ± 0.19	**Plaque** A= 0.18 ± 0.58 (-14.8%) C= 0.1 ± 0.53 (-6.5%) **Gingivitis** A= 0.06 ± 0.38 (-6%) C= - 0.08 ± 0.29 (-0.83%)	No significant difference between test and placebo groups for dental plaque or gingivitis.
Saxer et al. (2003) Germany	**A:** 2.68 ± 0.67 **C:** 2.52 ± 0.71	**A:** 2.43 ± 0.60 **C:** 2.67 ± 0.63	**A:** 2.69 ± 0.58 **C:** 2.17 ± 0.65	**A:** 1.93 ± 0.43 **C:** 1.64 ± 0.68	**Plaque** A= - 0.25 ± 0.89 (-9.3%) C= 0.15 ± 0.94 (-5.9%) **Gingivitis** A= -0.76 ± 0.72 (-28.2%) C= -0.53 ± 0.94 (-24.4%)	No significant difference between groups for dental plaque or gingivitis.
Grossman et al. (1989) USA	**A:** 1.49 ± 0.34* **B:** 1.41 ± 0.34* **C:**1.40 ± 0.34* **D:** 1.48 ± 0.34*	**A:** 1.31 ± 0.34* **B:** 0.76 ± 0.34* **C:**1.49 ± 0.34* **D:** 1.13 ± 0.34*	**A:** 0.54 ± 0.34* **B:** 0.53 ± 0.34* **C:**0.49 ± 0.34* **D:** 0.52 ± 0.34*	**A:** 0.35 ± 0.34* **B:** 0.25 ± 0.34* **C:**0.36 ± 0.34* **D:** 0.33 ± 0.34*	**Plaque** A= -0.18 ± 0.48 (-12%)† B= -0.65 ± 0.48 (-46%)†‡ C=0.09 ± 0.48 (-6.4%) D= -0.35 ± 0.48 (-23.6%)† **Gingivitis** A= -0.19 ± 0.48 (35.1%)† B= -0.28 ± 0.48 (52.8%)†‡ C=-0.13 ± 0.48 (26.5%) D= -0.19 ± 0.48 (-36.5%)†	CHX, EO and Veadent significantly better than placebo for gingivitis and dental plaque ; CHX significantly better than EO and Veadent and EO significantly better than Veadent for both dental plaque and gingivitis. .

∆, delta of mean (final – initial mean) and delta of standard deviation (SD); %, percentage of reduction; * imputed datum; CHX, chlorhexidine; EO, essential oils; + significant difference compared to positive control; † significant difference compared to placebo; ‡ significant difference compared to herbal product; - reduction, + accumulation/gain; π missing data (mean and interquartile range presented); A, herbal rinse; B, control rinse; C, placebo rinse; D, OE rinse.

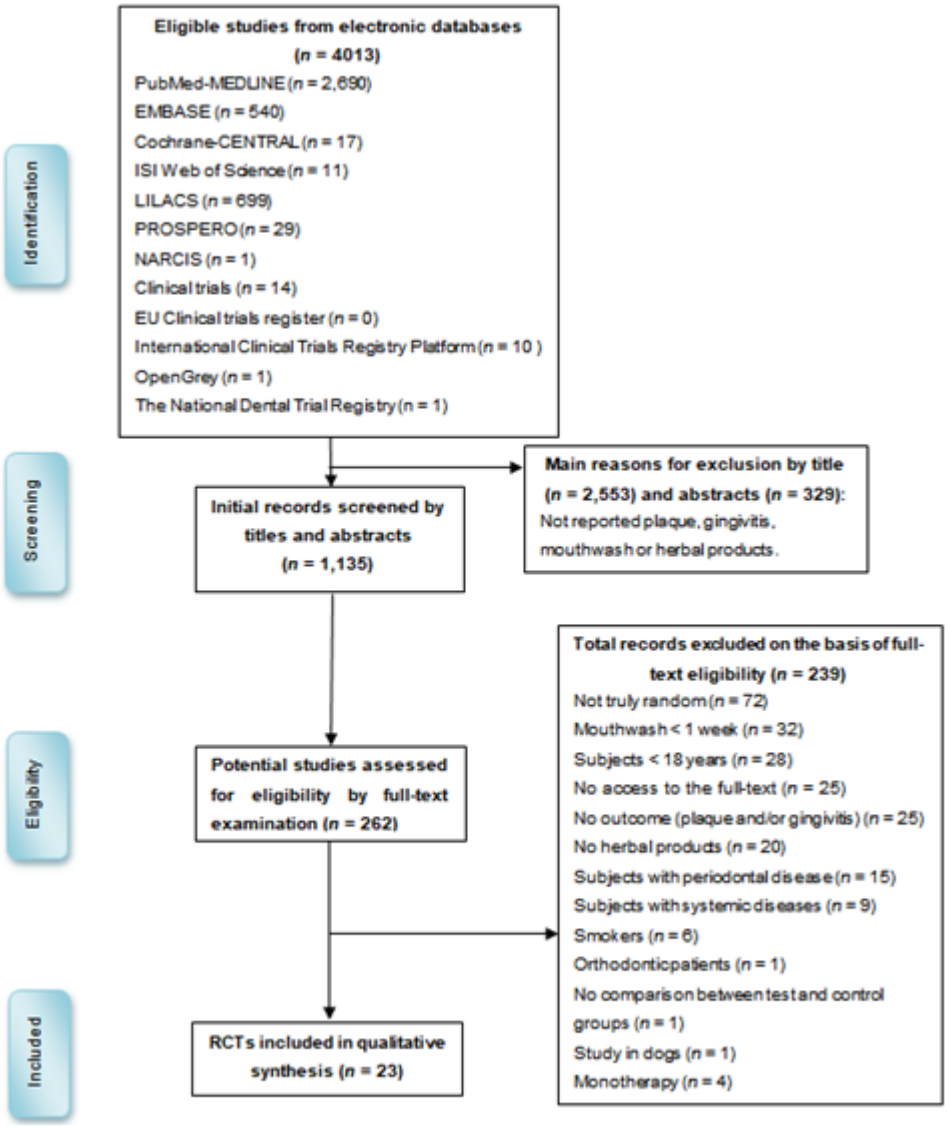

Fig 1. Selection of studies for systematic review

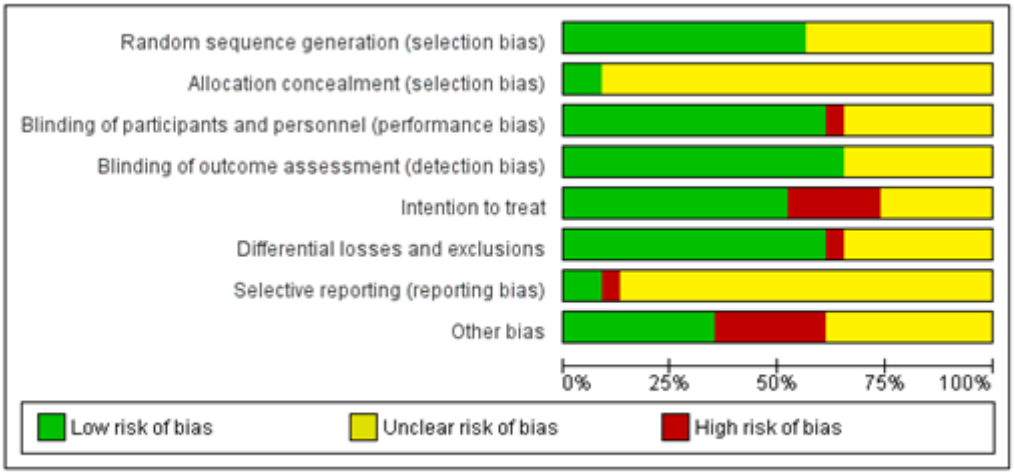

Fig 2. Graph of the bias risk of the selected studies

4 ARTIGO II - THE EFFECT OF HERBAL MOUTHWASHES ON HALITOSIS: A SYSTEMATIC REVIEW

Este artigo será submetido ao periódico *Journal of Clinical Periodontology*, ISSN: 1600-051X, Fator de impacto = 4.046; Qualis A1. As normas para publicação estão descritas no Anexo A.

The effect of herbal mounthrinse on halitosis: a systematic review

Running Title: Effect of herbal mouthwashes on halitosis

KEYWORDS: halitosis, phytotherapy, clinical trial, mouthrinse, systematic review

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Clinical Relevance

Scientific rationale for the study: The effect of herbal mouthrinses on halitosis remains unclear. A systematic review of randomized clinical trials was performed to determine the efficacy of herbal mouthrinses adjuvants to oral hygiene in individuals with halitosis.

Principal findings: significant differences were found favoring herbal mouthrinses over placebo solutions for all measures of halitosis. Sesame oil mouthrinse (*Sesamum indicum*) performed significantly better than chlorhexidine regarding self-rated halitosis. However, a moderate risk of bias was found and few studies have used the organoleptic method

Practical implications: Herbal mouthrinses demonstrated an effect on halitosis. However, studies with low risk of bias are necessary to confirm this effect.

Abstract

Aim: The aim of the present systematic review was to evaluate the effect of herbal mouthrinses in individuals with halitosis.

Material and Methods: Searches were conducted in the PubMed / MEDLINE, Cochrane - CENTRAL, EMBASE, Web of Science, LILACS / BIREME, Clinical Trials Registryand grey literature for articles published up to April, 2018. No language restriction was imposed.

Results: Among the sixty-two potentially eligible studies, four randomized clinical trials (RCT) were included in the present systematic review. Only one of the studies compared herbal products to chlorhexidine. The studies reported that the herbal mouthrinses achieved a significant reduction in halitosis compared to placebo mouthrinses. With regard to self-rated halitosis, the phytotherapeutic product containing sesame oil (*Sesamum indicum*) demonstrated a statistically greater reduction compared to 0.2% chlorhexidine. However, the methodological quality of the RCT was generally low, as the studies demonstrated a moderate risk of bias.

Conclusion: Despite the promising results of the herbal products compared to a placebo, high quality randomized clinical trials are needed to show evidence regarding the effect size and clinical relevance of these products for the recommendation of herbal mouthrinses. PROSPERO registry number: CRD42015015913

Introduction

Halitosis, oral malodor or bad breath is an unpleasant odor emanating from the oral cavity (Romano, Pigella, Guzzi, & Aimetti, 2010) that often causes embarrassment and can affect interpersonal social communication (De Geest, Laleman, Teughels, Dekeyser, & Quirynen, 2016; de Jongh, van Wijk, Horstman, & de Baat, 2016). According to epidemiological data, the prevalence of halitosis is around 31.8% (Silva et al., 2017) and the socioeconomic status of the country may exert an influence. Also, the prevalence seems to be higher among individuals with greater alcohol consumption and those with hyposalivation (Loesche & Kazor, 2002). Halitosis is currently one of the main reasons for seeking an oral health professional.

Approximately 87% of cases of halitosis have an oral etiology (Delanghe, Bollen, van Steenberghe, & Feenstra, 1998) and 4-8% can be attributed to non-oral causes (i.e., nasal inflammation, chronic tonsillitis, chronic sinusitis, diabetes mellitus) (Kapoor, Sharma, Juneja, & Nagpal, 2016). Volatile sulfur compounds (VSCs) are the main components of the malodor that emanates from the oral cavity. These compounds are produced by the metabolic bacterial degradation of food, tongue-coating debris, desquamated cells, saliva proteins, dental biofilm, gingival crevicular fluid and microbial decay (Ratcliff & Johnson, 1999).

The success of any halitosis intervention involves a reduction in VSC levels and other foul volatiles using mechanical and/or chemical measures. Mechanical interventions (i.e., dental hygiene and tongue scraping) reduce the number of VSC-producing bacteria, residual food matter and cellular debris from the teeth and tongue. However, mechanical methods seem to be limited with regard to reducing VSCs from others oral ecological regions (i.e., gums and mucous) (Deutscher, Derman, Barbe, Seemann, & Noack, 2018). A systematic review conducted by Outhouse et al. (2006) demonstrated that mechanical intervention with a tongue scraper alone seems to have very limited and short-lasting benefits, possibly due to its limited action in other niches of the oral cavity (Ayers & Colquhoun, 1998). In this context, mouthrinses may reach less accessible regions of the oral cavity and help to reduce the amount of VSCs (Ayers & Colquhoun, 1998).

Odor-masking agents in mouthrinses can be divided into those that affect the bacteria directly and those that neutralize the chemical compounds produced by bacteria (Carvalho, Tabchoury, Cury, Toledo, & Nogueira-Filho, 2004; Farrell, Baker, Somogyi-Mann, Witt, & Gerlach, 2006). Systematic reviews report that all mouthrinses

with active ingredients appear to be beneficial in reducing halitosis, especially those containing a combination of chlorhexidine, cetylpyridinium chloride and zinc (Blom, Slot, Quirynen, & Van der Weijden, 2012; Fedorowicz, Aljufairi, Nasser, Outhouse, & Pedrazzi, 2008).

Most commercial mouthrinses have formulations with alcohol and other industrial ingredients, the long-term use of which is known to have adverse effects (Loe, Schiott, Karring, & Karring, 1976; Van Strydonck, Slot, Van der Velden, & Van der Weijden, 2012). A recent systematic review of 12 case-control studies highlighted the association between an increased risk of head and neck cancer and excessive, high frequency or prolonged use of mouthrinses (Boffetta et al., 2016). However, as confounding variables and other forms of bias could not be excluded, these are not definitive findings. At the same time, they cannot be ignored. Thus, the lower cost and fewer side effects compared to conventional productshave placed herbal products in a prominent position in health care in recent years (Kothiwale, Patwardhan, Gandhi, Sohoni, & Kumar, 2014). Some herbs have been applied in periodontal therapy in an effort to improve the effectiveness of the home control of supragingival biofilm, gingival inflammation and halitosis (Ekor, 2014; Hamm, Gruber, & Willershausen, 1990; Li, Cai, & Wu, 1997).

To the best of our knowledge, there are no systematic reviews evaluated the efficacy of herbal products for the control of halitosis. Therefore, the aim of the present study was to perform a systematic review to investigate the effect of herbal formulations compared to active ingredients and placebo mouthrinses in controlling halitosis in healthy individuals.

Material and methods

This study was conducted according to the Cochrane Handbook (Higgins & Green, 2011) and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009) .

Focused question (PICOS)

Based on randomized controlled trials involving systemically healthy individuals with a diagnosis of halitosis: what is the effect of herbal mouthrinses on oral malodor compared to mouthrinse with an active ingredient or placebo?

Search strategy

We conducted an electronic search of the PubMed/MEDLINE, Cochrane-CENTRAL, EMBASE (Excerpta Medical Database by Elsevier), TRIP, ISI Web of Science, China National Knowledge Infrastructure (CNKI), and LILACS (Latino-American and Caribbean Literature in Health Science) databases to identify literature published up to April 3rd, 2018. The search involved a combination of controlled vocabulary and text words based on the search strategy for the PubMed/MEDLINE database listed in Table 1. A similar search strategy was adapted for the other databases. We searched for relevant ongoing trials from the US Clinical Trials Register (http://www.clinicaltrials.gov). We also searched the grey literature (OpenGrey, CAPES thesis bank) and manually searched the reference lists of all full texts of interest. No restriction was imposed with regard to language. The Mendeley Desktop 1.17.13 reference manager (England) was used to group and manage the references.

Eligibility criteria

- Study design: Randomized controlled clinical trials (no language restrictions);
- Participants: Healthy individuals, ≥ 18 years old, with clinical or self-assessed diagnosis of halitosis;
- Intervention: Herbal mouthrinse used for at least one 1 week;
- Comparison: Placebo rinse or control rinse ;
- Outcomes: Improvement in halitosis assessed by at least one of the following: organoleptic score, portable H_2S monitor (Halimeter®, Chatsworth, Los Angeles, CA), gas chromatography (GC) or self-perceived halitosis (SPH).

Screening and selection

Two trained reviewers (MC and APG) independently screened all titles and abstracts for eligible papers. Papers without abstracts, but with titles suggesting a relation to the objectives of the review were also pre-selected and submitted to the full-text analysis for eligibility. The full texts of the pre-selected articles were read in detail by the same reviewers (MC and APG) (*Kappa agreement* = 0.90).

Papers that fulfilled all of the selection criteria were processed for data extraction (*K*= 0.93). Duplicates were excluded. Studies that fulfilled the eligibility criteria were included in the present review. Divergences of opinion between the

reviewers were resolved by a discussion. If a disagreement persisted, the judgment of a third reviewer (FBZ) was considered decisive. When necessary, the authors of the selected articles were contacted to clarify any ambiguities and provide missing data.

Data extraction

The following information was extracted: (1) Study data: (A) author, year of publication, country, (B) design (parallel or cross) and length of follow-up; (2) Participants: (A) sample size, (B) age and (C) sex; (3) Intervention and control: (A) scientific name, common name andcommercial label (4) Regimen: dose (milliliter - ml), number of times per day, time of use, coadjuvant oral hygiene; (5) Inclusion and exclusion criteria; (6) Results: (A) Criteria for halitosis evaluation, (B) baseline and final measurements. In cases of missing data, e-mails were sent to the authors for clarification. If no response was obtained, the data were extracted from graphs and figures with the aid of the WebPlotDigitizer 3.8 (Copyright ©, Austin, Texas, USA).

Data analysis

After a preliminary evaluation of the selected papers, considerable heterogeneity was found in the study designs, characteristics, outcome variables and results. It was therefore not possible to perform a valid quantitative analysis of the data and subsequent meta-analysis. Therefore, the data are presented descriptively.

Risk of bias (quality) assessment

The quality of the studies included in the present review was appraised by two reviewers (APG and MC) (K = 0.98) using a bias appraisal method specific to the study design (Cochrane Handbook for Systematic Reviews of Interventions 5.0.1; Higgins & Green, 2011). The criteria were adapted and divided into seven principal domains. For each study, the criteria were classified as exhibiting a low, high or unclear (without information or uncertain) risk of bias. For the final classification of the risk of bias, divergences of opinion between the reviewers were resolved by consensus.

Results

Study selection

The searches resulted in the identification of 1272 studies after the removal duplicate papers. The screening of the titles and abstracts led to the pre-selection of 62 articles for submission to full-text analysis. The reference lists of the pre-selected papers did not reveal any additional suitable papers. After the analysis of the pre-

selected papers, four met the eligibility criteria and were included in the qualitative synthesis. Figure 1 shows the study selection process and reasons for exclusion in each step.

Assessment of heterogeneity

Detailed information on the characteristics of the studies, authors' conclusions and statistical significance between the groups are presented in Tables 2, 3 and 4.

Table 2 shows the characteristics of the populations studied in each randomized controlled trial. In the studies by Rassameemasmaung et al. (2013), Satthanakul et al. (2015) and Rassameemasmaung et al. (2007), the age ranges were 18 to 55 years, 23 to 40 years and 17 to 37 years, respectively. Regarding the clinical characteristics, Asokan et al. (2011), Rassameemasmaung et al. (2013) and Rassameemasmaung et al. (2007) included individuals with gingivitis, whereas this information is not clear in the study by Satthanakul et al. (2015). It is possible that individuals with periodontitis were not included in the studies by Asokan et al. (2011) and Satthanakul et al. (2015). However, there is no clear information on the exclusion of patients with periodontitis in any study. Asokan et al. (2011), Rassameemasmaung et al. (2013) and Rassameemasmaung et al. (2007) excluded smokers.

Considerable clinical heterogeneity was found among the interventions. Asokan et al. (2011) compared the effect of "oil pulling"[1] with sesame oil (*Sesamum indicum*) to 0.2% chlorhexidine mouthrinse. The organoleptic test and self-assessments of breath were performed at baseline and after 14 days. A reduction in the organoleptic score and self-rated halitosis occurred in both the test and control groups, with significant differences favoring the test group only for the self-rated breath score.

Rassameemasmaung et al. (2013) compared green tea (*Camellia sinensis)* to a placebo. Individuals with gingivitis and who had more than 80 parts per billion of VSCs in the morning breath were randomly assigned to the green tea or placebo mouthrinse group. VSCs were recorded at baseline and after 28 days of use of the products. VSCs were reduced by 38.61% in the green tea group and 10.86% in the placebo group, with a significant difference favoring the green tea group.

Satthanakul et al. (2015) compared the efficacy of a lemongrass oil[2] mouthrinse and placebo mouthrinse. Twenty healthy subjects were randomly allocated to the

[1] For oil pulling or oil swishing therapy, a tablespoon of sesame oil is taken in the mouth, sipped, sucked, and pulled between the teeth for 10 to 15 min until the viscous oil turns thin and milky white. This procedure should be followed by tooth brushing.
[2] *Cymbopogon citratus* Stapf is a volatile oil obtained from lemongrass leaves.

different groups. Halitosis was assessed based on the organoleptic score and VSCs on days 0 and 8 for each volunteer. The results showed that the lemongrass mouthrinse was more effective at reducing VSCs and the organoleptic score after eight days, with significant differences favoring the lemongrass group for both outcomes.

Rassameemasmaung et al., (2007) evaluated sixty healthy subjects with halitosis after 15 days of use of a mouthrinse containing extract of G*arcinia mangostana* L and a placebo solution. A reduction of VSC level was found in the intra-group analyses in all periods ($P < 0.05$). The extract of G*arcinia mangostana* L mouthrinse reduced VSC levels by 59.68% from baseline (248.35 ± 172.30) to the 15-day evaluation (100.54 ± 69.37) ($P < 0.05$). The reduction in the placebo group was 25.74% (237.45 ± 114.15 to 176.83 ± 123.6) ($P < 0.05$).

Characteristics of the halitosis assessments

Asokan et al. (2011) used the organoleptic score. Rassameemasmaung et al. (2013), Satthanakul et al. (2015) and Rassameemasmaung et al. (2007) measured VSCs. Asokan et al. (2011) and Satthanakul et al. (2015) used self-assessments.

Quality assessment

Figure 2 displays detailed information on the methodological quality and risk of bias of the studies. Most studies had a moderate risk of bias in both domains. A high risk of selection bias was found

Discussion

This present review demonstrated that few randomized clinical trials have been conducted on the effect of herbal mouthrinse for the control of halitosis. Among the four studies selected for analysis, the herbal products led to a significant reduction in halitosis in comparison to placebo solutions. Moreover, sesame oil (*Sesamum indicum*) achieved a better result than 0.2% chlorhexidine based on the self-reported method.

Sesamum indicum has been widely used as a folk medicine in India to avoid oral malodor and gingival bleeding (Asokan et al., 2011). However, its effect on the reduction in halitosis was only significant in the self-assessments. In the studies

conducted by Rassameemasmaung et al. (2007 and 2013), the herbal products *G. mangostana* (mangosteen) and *Camellia sinensis* (green tea) achieved statistically significant reductions in VSCs measured using a halimeter compared to placebo solutions. Satthanakul et al. (2015) found a significantly better effect on halitosis, evaluated using the organoleptic method, with a lemongrass oil mouthrinse. The findings demonstrate that the herbal products tested (*G. mangostana, Cymbopogan citratus* and *Camellia sinensis*) were significantly better at reducing halitosis than placebo solutions. However, the lack of an organoleptic evaluation for two of these herbal mouthrinse could be a relevant point.

Previous studies report that the organoleptic method performed by a trained examiner is considered the gold standard and most reliable evaluation of halitosis (Rosenberg et al., 1995). However, this claim has been contested by some authors, who have demonstrated that measurements using a halimeter seem to be more reproducible, although they may be less reliable than the organoleptic method (Silwood, Grootveld, & Lynch, 2001).

All the randomized clinical trials included in the present review had a short to medium evaluation period of seven (Satthanakul et al., 2015), 14 (Asokan et al., 2011; Rassameemasmaung et al., 2007) or 28 days (Rassameemasmaung et al., 2013). Although mouthrinses are used and prescribed for short periods, the American Dental Association stipulates a study period of six months for the evaluation of the effectiveness and safety of mouthrinses (American Dental Association, 2008). Regarding the outcome, there are currently no standardized and accepted protocols for the treatment of halitosis and there is no evidence that such treatment requires a minimum period of action of the mouthrinses (Morita & Wang, 2001).

The present study has limitations that should be addressed. The methodological quality of the studies analyzed was generally low, with a moderate risk of bias, and the no standardization was found in the diagnosis of halitosis. Self-rated halitosis and the use of a halimeter are not as reliable as the organoleptic method. Moreover, no studies involved the concomitant removal of coated tongue with the interventions described and only Rassameemasmaung et al. (2007) offered an evaluation of adverse effects. The findings demonstrate the need for further studies on the evaluation of halitosis using the organoleptic method, which is considered the gold standard.

Conclusion

The findings of the present review demonstrate that herbal mouthrinses achieve better results than placebo solutions regarding a reduction in halitosis. Moreover, a sesame oil-based mouthrinse exhibited a stronger size effect compared to chlorhexidine. However, the risk of bias in the majority of studies does not enable drawing definitive conclusions. Therefore, further randomized clinical trials should be conducted with better methodological quality and involving evaluations with the organoleptic method to strengthen the evidence regarding the effect of herbal mouthrinses.

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References

American Dental Association. (2008) Acceptance Program Guidelines: Chemotherapeutic Products for Control of Gingivitis. Council on Scientific Affairs, Available at: http://www.ada.org/ en/science-research/ada-seal-of acceptance/how-to-earn-the-ada-seal/ guidelines-for-product-acceptance

Asokan, S., Emmadi, P., Sivakumar, N., Kumar, Rs., & Raghuraman, R. (2011). Effect of oil pulling on halitosis and microorganisms causing halitosis: A randomized controlled pilot trial. *Journal of Indian Society of Pedodontics and Preventive Dentistry*, *29*(2), 90. https://doi.org/10.4103/0970-4388.84678

Ayers, K. M., & Colquhoun, A. N. (1998). Halitosis: causes, diagnosis, and treatment. *The New Zealand Dental Journal*, *94*(418), 156–60. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9889536

Blom, T., Slot, D., Quirynen, M., & Van der Weijden, G. (2012). The effect of mouthrinses on oral malodor: a systematic review. *International Journal of Dental Hygiene*, *10*(3), 209–222. https://doi.org/10.1111/j.1601-5037.2012.00546.x

Boffetta, P., Hayes, R. B., Sartori, S., Lee, Y.-C. A., Muscat, J., Olshan, A., … Hashibe, M. (2016). Mouthwash use and cancer of the head and neck. *European Journal of Cancer Prevention*, *25*(4), 344–348.

Carvalho, M. D., Tabchoury, C. M., Cury, J. A., Toledo, S., & Nogueira-Filho, G. R. (2004). Impact of mouthrinses on morning bad breath in healthy subjects. *Journal of Clinical Periodontology*, *31*(2), 85–90. https://doi.org/10.1111/j.0303-6979.2004.00452.x

Cheraskin, E., & Ringsdorf, W. M. J. (1982). Tissue tolerance to orthodontic banding. A study in carbohydrate metabolism. *The Angle Orthodontist*, *52*(2), 118–128. Clinical Trial, Comparative Study, Controlled Clinical Trial, Journal Article. https://doi.org/10.1043/0003-3219(1982)052<0118:TTTOB>2.0.CO;2

De Geest, S., Laleman, I., Teughels, W., Dekeyser, C., & Quirynen, M. (2016). Periodontal diseases as a source of halitosis: a review of the evidence and

treatment approaches for dentists and dental hygienists. *Periodontology 2000*, *71*(1), 213–227. Journal Article, Review. https://doi.org/10.1111/prd.12111

de Jongh, A., van Wijk, A. J., Horstman, M., & de Baat, C. (2016). Self-perceived halitosis influences social interactions. *BMC Oral Health*, *16*(1), 1–7. https://doi.org/10.1186/s12903-016-0189-9

Delanghe, G., Bollen, C., van Steenberghe, D., & Feenstra, L. (1998). [Halitosis, foetor ex ore]. *Nederlands tijdschrift voor tandheelkunde*, *105*(9), 314–317. English Abstract, Journal Article.

Deutscher, H., Derman, S., Barbe, A. G., Seemann, R., & Noack, M. J. (2018). The effect of professional tooth cleaning or non-surgical periodontal therapy on oral halitosis in patients with periodontal diseases. A systematic review. *International Journal of Dental Hygiene*, *16*(1), 36–47. Journal Article, Review. https://doi.org/10.1111/idh.12306

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Neurology*, *4 JAN*(January), 1–10. https://doi.org/10.3389/fphar.2013.00177

Farrell, S., Baker, R. A., Somogyi-Mann, M., Witt, J. J., & Gerlach, R. W. (2006). Oral malodor reduction by a combination of chemotherapeutical and mechanical treatments. *Clinical Oral Investigations*, *10*(2), 157–163. https://doi.org/10.1007/s00784-006-0044-5

Fedorowicz, Z., Aljufairi, H., Nasser, M., Outhouse, T. L., & Pedrazzi, V. (2008). Mouthrinses for the treatment of halitosis. In Z. Fedorowicz (Ed.), *Cochrane Database of Systematic Reviews* (p. CD006701). Chichester, UK: John Wiley & Sons, Ltd. https://doi.org/10.1002/14651858.CD006701.pub2

Hamm, G., Gruber, I., & Willershausen, B. (1990). [Influence of conservative periodontal treatment on degree of gingival inflammation]. *ZWR*, *99*(11), 877–878. English Abstract, Journal Article.

Higgins, J. P., & Green, S. (n.d.). Cochrane Handbook for Systematic Reviews of Interventions Cochrane Book Series THE COCHRANE COLLABORATION ®. https://pdfs.semanticscholar.org/4b43/91c08c45ebfcd046a53106c97ca09fcdf9fa.pdf

Kapoor, U., Sharma, G., Juneja, M., & Nagpal, A. (2016). Halitosis: Current concepts on etiology, diagnosis and management. *European Journal of Dentistry*, *10*(2), 292. https://doi.org/10.4103/1305-7456.178294

Kothiwale, S., Patwardhan, V., Gandhi, M., Sohoni, R., & Kumar, A. (2014). A comparative study of antiplaque and antigingivitis effects of herbal mouthrinse containing tea tree oil, clove, and basil with commercially available essential oil mouthrinse. *Journal of Indian Society of Periodontology*, *18*(3), 316. https://doi.org/10.4103/0972-124X.134568

Li, X. C., Cai, L., & Wu, C. D. (1997). Antimicrobial compounds from Ceanothus americanus against oral pathogens. *Phytochemistry*, *46*(1), 97–102. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9276981

Lobene, R. R., Kashket, S., Soparkar, P. M., Shloss, J., & Sabine, Z. M. (1979). The effect of cetylpridinium chloride on human plaque bacteria and gingivitis. *Pharmacology and Therapeutics in Dentistry*, *4*(1), 33–47. Journal Article, Research Support, U.S. Gov't, P.H.S.

Loe, H., Schiott, C. R., Karring, G., & Karring, T. (1976). Two years oral use of chlorhexidine in man. I. General design and clinical effects. *Journal of Periodontal Research*, *11*(3), 135–144. Clinical Trial, Journal Article, Research Support, U.S. Gov't, Non-P.H.S.

Loesche, W. J., & Kazor, C. (2002). Microbiology and treatment of halitosis. *Periodontology 2000*, *28*, 256–79. http://www.ncbi.nlm.nih.gov/pubmed/12013345

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & PRISMA Group. (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Medicine*, *6*(7), e1000097.

Morita, M., & Wang, H. L. (2001). Association between oral malodor and adult periodontitis: a review. *Journal of Clinical Periodontology*, *28*(9), 813–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11493349

Outhouse, T. L., Fedorowicz, Z., Keenan, J. V, & Al-Alawi, R. (n.d.). A Cochrane systematic review finds tongue scrapers have short-term efficacy in controlling halitosis. *General Dentistry*, *54*(5), 352–9; 360, 367–8; quiz 360. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17004573

Rassameemasmaung, S., Phusudsawang, P., & Sangalungkarn, V. (2013). Effect of green tea mouthwash on oral malodor. *ISRN Preventive Medicine*, *2013*, 975148. Journal Article. https://doi.org/10.5402/2013/975148

Rassameemasmaung, S., Sirikulsathean, A., Amornchat, C., Hirunrat, K., Rojanapanthu, P., & Gritsanapan, W. (2007). Effects of herbal mouthwash containing the pericarp extract of Garcinia mangostana L on halitosis, plaque and papillary bleeding index. *Journal of the International Academy of Periodontology*, *9*(1), 19–25.

Ratcliff, P. A., & Johnson, P. W. (1999). The relationship between oral malodor, gingivitis, and periodontitis. A review. *Journal of Periodontology*, *70*(5), 485–489. Journal Article, Research Support, Non-U.S. Gov't, Research Support, U.S. Gov't, P.H.S., Review. https://doi.org/10.1902/jop.1999.70.5.485

Romano, F., Pigella, E., Guzzi, N., & Aimetti, M. (2010). Patients' self-assessment of oral malodour and its relationship with organoleptic scores and oral conditions. *International Journal of Dental Hygiene*, *8*(1), 41–46. Comparative Study, Journal Article. https://doi.org/10.1111/j.1601-5037.2009.00368.x

Rosenberg, M., Kozlovsky, A., Gelernter, I., Cherniak, O., Gabbay, J., Baht, R., & Eli, I. (1995). Self-estimation of Oral Malodor. *Journal of Dental Research*, *74*(9), 1577–1582. https://doi.org/10.1177/00220345950740091201

Satthanakul, P., Taweechaisupapong, S., Paphangkorakit, J., Pesee, M., Timabut, P., & Khunkitti, W. (2015). Antimicrobial effect of lemongrass oil against oral malodour micro-organisms and the pilot study of safety and efficacy of lemongrass mouthrinse on oral malodour. *Journal of Applied Microbiology*, *118*(1), 11–17. https://doi.org/10.1111/jam.12667

Silva, M. F., Leite, F. R. M., Ferreira, L. B., Pola, N. M., Scannapieco, F. A., Demarco, F. F., & Nascimento, G. G. (2017). Estimated prevalence of halitosis: a systematic review and meta-regression analysis. *Clinical Oral Investigations*. https://doi.org/10.1007/s00784-017-2164-5

Silwood, C. J., Grootveld, M. C., & Lynch, E. (2001). A multifactorial investigation of the ability of oral health care products (OHCPs) to alleviate oral malodour. *Journal of Clinical Periodontology*, *28*(7), 634–41. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11422584

Van Strydonck, D. A. C., Slot, D. E., Van der Velden, U., & Van der Weijden, F. (2012). Effect of a chlorhexidine mouthrinse on plaque, gingival inflammation and staining in gingivitis patients: a systematic review. *Journal of Clinical Periodontology*, *39*(11), 1042–1055. Journal Article, Research Support, Non-U.S. Gov't, Review. https://doi.org/10.1111/j.1600-051X.2012.01883.x

Table 1. Search strategy used in PubMed/MEDLINE

	Searchterms
No. 4	No 1 and No 2 and No 3
No. 3 TypeofStudy	((clinical[Title/Abstract] AND trial[Title/Abstract]) OR clinical trials as topic[MeSH Terms] OR clinical trial[Publication Type] OR random*[Title/Abstract] OR random allocation[MeSH Terms] OR therapeutic use[MeSH Subheading])
No. 2 Outcome	"Halitosis"[Mesh] OR "halitosis*" OR] "halitoses" OR "dimethyl sulfide" [Supplementary Concept] OR "dimethyl sulfide" OR "dimethylamine" [Supplementary Concept] OR "dimethylamine" OR "trimethylamine" [Supplementary Concept] OR "trimethylamine" OR "oral malodor" OR "halimetry" OR "breath odor" OR "bad breath" OR "ore breath" OR "Carbon disulphide" [Mesh] OR "carbon disulfide" OR "Acetone" [Mesh] OR "acetone" OR "morning halitosis" OR "volatile sulphur compounds" [All Fields] OR "sulfur compounds" [Mesh] OR "sulfur compounds" OR "sulfhydryl compounds" [Mesh] OR "sulfhydryl compounds" OR "Compounds, Sulfhydryl" OR "Thiols" OR "Mercaptans" OR "Mercapto Compounds" OR "Compounds, Mercapto" OR "Foetor ex ore breath" OR"sulfides" [Mesh] OR "sulfide*" OR "allyl methyl sulfide" [Supplementary Concept] OR "allyl methyl sulfide"
No. 1 Intervention	"Drugs, Chinese Herbal" [Mesh] OR "Chinese Drugs, Plant" OR "Chinese Herbal Drugs" OR "Herbal Drugs, Chinese" OR "Plant Extracts, Chinese" OR "Chinese Plant Extracts" OR "Extracts, Chinese Plant" OR "Herbal Medicine" [Mesh] OR "Medicine, Herbal" OR "Herbalism" OR "Phytotherapy" [Mesh] OR "Phytotherapy" OR "Herbal Therapy" OR "Herb Therapy" OR "Plants, Medicinal" [Mesh] OR "Plant Extracts" [Mesh] OR "Herbal*" AND "Mouthwashes" [Mesh] OR "Mouthwashe*" OR "mouthwashes" [Pharmacological Action] OR "Mouthrinse" OR "Mouthrinses" OR "Rinse*" OR "Oral Rinse"

Table 2 . General characteristics of selected studies (n = 4)

Author Year Country	Design / duration	Number of participants	N (%) gender / Mean age (SD)	Intervention (scientific name, common name, manufacturer, formulation)	Regimen	Inclusion criteria	Exclusion criteria
Asokan et al. (2011) India	Parallel 14 days	**A**: 10 **B**: 10	**N (%) gender** NR **Mean age (SD)** **A**:18 **B**:18	**A:***Sesamum Indicum* – sesame oil (Idhayam Oil, VVV Sons India); (NR) **B:** 0.2% CHX (Hexidine, ICPA Health Products Ltd, India);	1 x/day 15 ml 60'' Adjuvant	(1) Healthy individuals 17 to 19 years of age;(2) at least 24 permanent teeth with gingival probing depth < 3 mm; (3) plaque index = 1 in more than 10% of sites	(1) Use of antibiotics in previous 3-4 weeks; (2) Use of orthodontic appliance, dentures;(3) smokers
Rassameemasmaung et al. (2013) Thailand	Parallel 28 days	**A:** 30 **B**: 30	**N (%) gender** 3♂27♀ **Mean age (SD)** **A:** 27.2 ± 9.9 **B:** 25.8 ± 7.6	**A:** *Camellia sinensis* (green tea) (NR) **B:** Placebo	2x/day 15 ml 60'' Adjuvant	(1) At least 20 teeth (2) VSCs more than 80 parts per billion in breath	(1) Complicating systemic factors, (2) oral mucosa lesions, (3) smokers, (4) use of antibiotic in previous month
Satthanakul et al. (2015) Thailand	Parallel 7 days	**A/B:** 20	**N (%) gender** 5♂15♀ **Mean age (SD)** **A**:21 **B**:21	**A:** *Cymbopogon citratus* (lemon grass oil)-5% (LG) (Thai China Flavours and Fragrances Industry Co. (Bangkok, Thailand); **B:** Placebo	2x/day 15 ml 60'' Adjuvant	1) Good health, with no history of serious medical conditions or allergy to LG. (2) Not pregnant or lactating (3) No clinical signs of oral disease based on visual examination by dentist	NR
Rassameemasmaung et al. (2007) Thailand	Crossover 14 days	**A:** 30 **B:** 30	**N (%) gender** 12♂48♀ **Mean age (SD)** **A**: 26.15 ± 6.25 **B**: 26.15 ± 6.25	**A:** *G. mangostana* (mangosteen) (NR) **B:** Placebo	2x/day 15 ml 60'' Adjuvant	(1) At least 20 teeth, mild to moderate chronic gingivitis (gingival index of each individual tooth 1-2 according to Löe and Silness, 1963) (2) VSCs more than 80 parts per billion in breath during morning breath	(1) Smokers; (2) denture wearers; (3) systemic factors; (4) oral disease (5) use of antibiotic in previous month

Legend: CHX, chlorhexidine; NR, not reported; ♂, female; ♀, male, SD, standard deviation, LG, lemongrass oil; VSCs, volatile sulfur compounds

Table 3. Criteria for assessment of halitosis and outcome measures as baseline and end of study (n = 4)

Author Year	Recommendations before assessment of halitosis	Assessment of halitosis	Baseline and final measures		Author' conclusions
			Test	Control	
Asokan et al. (2011)	Previous night: participants instructed not to ingest spicy foods, garlic, onions or alcoholic beverages and perform tooth brushing before 12 a.m. Morning of exam, absolute fasting with no oral hygiene measures and no use of fragranced cosmetics/perfumes.	**Organoleptic score** Participants instructed to maintain mouth completely closed for 3 minutes, breathing only through nose, then slowly release breath through the mouth at a distance of 19 cm from examiner's nose; Intensity scores: 0 = no odor 1 = nearly imperceptible odor 2 = mild odor, but clearly perceptible 3 = moderate odor 4 = strong offensive odor 5 = extremely strong odor **Self-report** Participants instructed to lick their wrist and smell after drying. Results expressed same as organoleptic score	Significant reductions in organoleptic and self-rated scores in both groups; no significant difference between groups. Significantly greater reduction in test group only for self-rated halitosis		Significant difference between CHX and sesame oil mouthwash favoring sesame oil in self-rated halitosis
Rassameemasmaung et al. (2013)	Participants instructed to refrain from brushing teeth, eating or drinking at least two hours prior to reading; rinse mouth with water at least 20 minutes prior to reading to avoid dry mouth effect and not speak for at least 5 min. Screening test performed between 7:00/8:30 a.m.	**- Halimeter (VSC)** (Model RH-17, Interscan Corp, CA, USA); set of three 30-sec readings performed	B: 187.7 (90.3) F: 105.5 (66.6)	B: 185.3 (115.1) F: 162.4(115.7)	Green tea significantly better than placebo at reducing VSCs.
Satthanakul et al. (2015)	VSCs measured at 8 a.m. prior to brushing teeth	**- Halimeter (VSC)** (Model RH-17, Interscan Corp, CA, USA); set of three 30-sec readings performed **Self-report** After waking in morning, participants instructed to assess breath daily using the following scale: 1 = most pleasant 2 = very pleasant 3 = moderately pleasant 4 = slightly pleasant	**VSCs** B: 62.8 (7.5) F: 32.1* **Self-assessment** B: 5.9 (0.6) F: 7.5 (1.5)	**VSC** B: 57.7 (8.4) F: 6.5(10.5)* **Self-assessment** B: 6 (0.7) F: 8.1 (1.5)	LG significantly better than placebo for both halimeter findings and self-assessments .

		5 = neither pleasant nor unpleasant 6 = a little unpleasant 7 = moderately unpleasant 8 = very unpleasant 9 = most unpleasant			
Rassameemasmaung et al. (2007)	Screening tests performed at 8 a.m. Participants instructed to refrain from brushing teeth, eating, drinking and chewing gum at least 2 hours prior to reading; rinse with water 20 minutes prior to test to avoid dry mouth effect and not to speak for at least 5 minutes prior to onset of test.	**- Halimeter (VSC)** (Model RH-17, Interscan Corp, CA, USA); set of three 30-sec readings performed.	B: 248.35 (172.30) F: 100.54 (69.37)	B: 237.45 (114.15) F: 176.83 (123.6)	*G. mangostana* (mangosteen) significantly better than placebo in assessment of VSCs

B, baseline; F, final;* determined graphically; VSCs, volatile sulfur compounds; NR, not reported; LG, lemongrass oil; CHX, chlorhexidine

Table 4. Descriptive summary of statistical significance between groups

Reference	Intervention	Comparison	Organoleptic score	VSC	Self-report
Asokan et al. (2011)	Sesame oil	0.2% chlorhexidine	0	□	+
Rassameemasmaung et al. (2013)	Green tea	Placebo	□	+	□
Satthanakul et al. (2015)	Lemongrass oil	Placebo	□	+	+
Rassameemasmaung et al. (2007)	Pericarp extract of *G. mangostana*	Placebo	?	+	?

+, Significant difference in favor of test group; 0, no significant difference between test and control groups; ?, inconclusive data which does not allow conclusions concerning statistical significance; □, no data available regarding this outcome

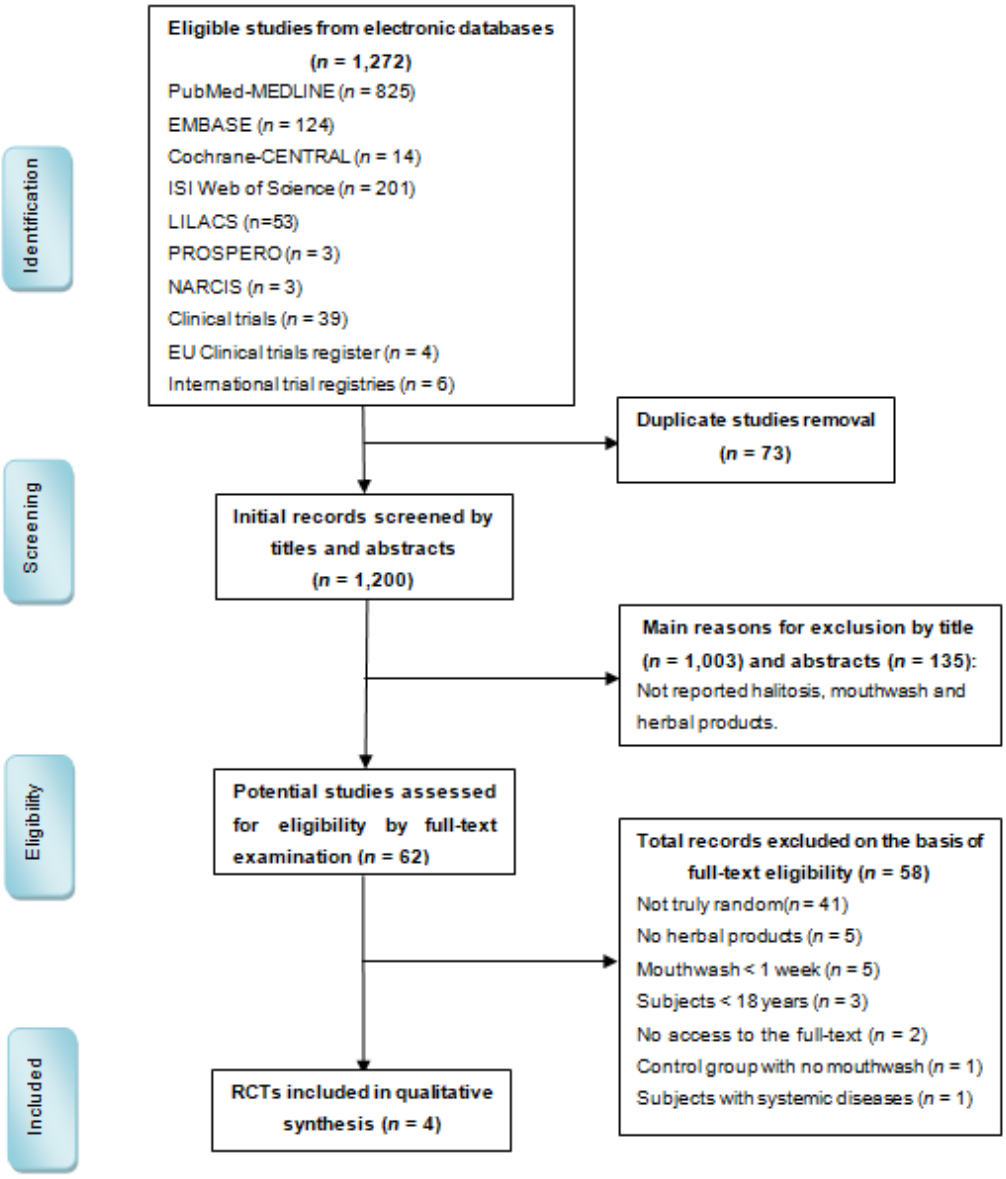

Fig. 1. Selection of studies for systematic review.

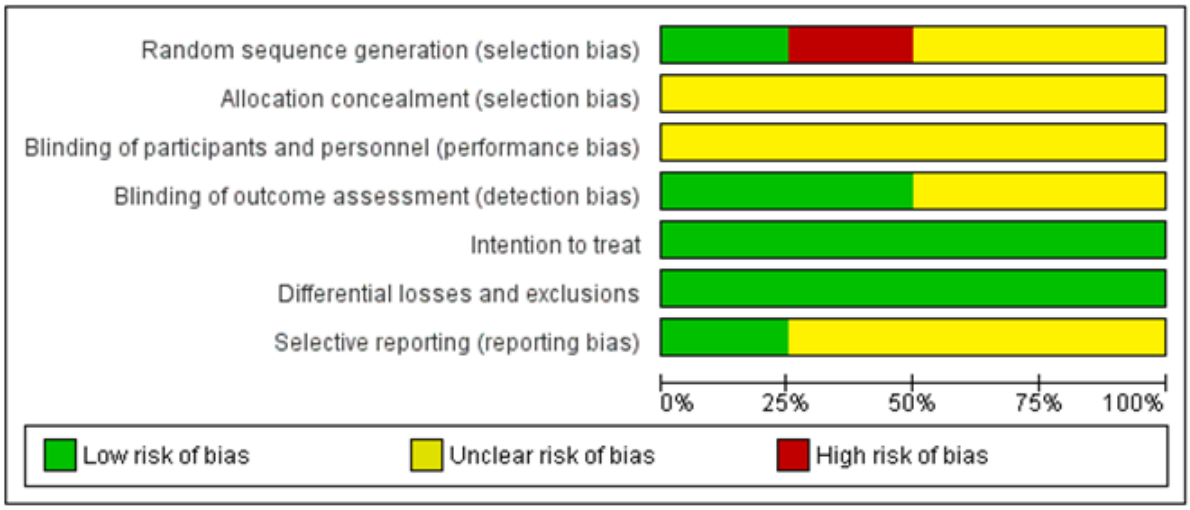

Fig. 2. Graph of the bias risk of the selected studies.

5 DISCUSSÃO

Dados sobre as doenças periodontais revelam uma alta prevalência populacional (SUSIN et al., 2004; MURRAY et al. 2015; FUNIERU et al., 2017), sendo a gengivite a doença mais prevalente no ser humano (GUINNESS WORLD RECORDS 2001). Embora saibamos que é perfeitamente possível alcançar níveis adequados de saúde gengival, apenas com controle mecânico supragengival associado a dentifrícios, esses dados denotam que a qualidade deste controle, em nível populacional, é bastante deficiente (VAN DER WEIJDEN; HIOE, 2005).

Este panorama epidemiológico dá espaço para a pesquisa de produtos/formulações que possam complementar os métodos mecânicos na busca de um melhor controle de biofilme, de inflamação gengival e de halitose (BATTINO et al., 2002; AYERS; COLQUHOUN, 1998). É bem verdade que empresas que trabalham com produtos na linha de cuidados bucais também se valem desta espaço para alavancar vendas, tendo em vista a diversidade de antissépticos comercialmente disponíveis. Embora o apelo e a força de marketing sugiram resultados fantásticos para cada um dos produtos, dados científicos revelam que apenas poucos deles trarão resultados melhores, ainda com relevância clínica questionável. Além disso, estes produtos podem exibir efeitos adversos, como a clorexidina que produz manchamento dental se usada por períodos prolongados (DEUTSCHER et al., 2017; VAN STRYDONCK et al., 2012) e os óleos essenciais (Listerine®) que apresentam sensação de ardência na cavidade bucal (MOREIRA et al., 2009). Paralelamente, há uma tendência mercadológica de aumento nas vendas de produtos naturais, sem a inserção de princípios ativos quimicamente desenvolvidos e/ou com álcool na formulação, o que tem colocado os produtos fitoterápicos em lugar de destaque nos cuidados com a saúde nos últimos anos (BUCHAUL, 2001).

Nossos dados mostraram que os produtos ervais testados resultaram em reduções significativas em biofilme dental, inflamação gengival e halitose quando comparados a placebo. Quando comparados a CHX, a magnitude da redução percentual dos desfechos foi promissora a favor de algumas ervas (*Camelia sinensis, Azadirachta indica, Anacardium occidentale Linn, Curcuma longa e Sesamum indicum*).

Estudos com *Camellia sinensis* (Chá verde) demonstraram uma redução de biofilme dental e inflamação gengival semelhante a CHX (PRIYA et al.,2015; BALAPPANAVAR et al., 2013). Entretanto, no estudo de Balappanavar et al., (2013), a CHX foi usada por duas semanas e o chá verde por três semanas. Assim, as diferenças a favor do produto erval podem ser explicadas, pelo menos em parte, por essas diferenças metodológicas. Esse efeito da *Camellia sinensis* também foi encontrado por Priya et al., (2015) e Rassameemasmaung et al. (2013) que encontraram reduções percentuais de biofilme dental comparado a CHX e placebo, respectivamente.

Já efeitos semelhantes a CHX na redução da inflamação gengival foram observadas para as soluções com as ervas *Anacardium occidentale Linn (*56,6%) (GOMES et al., 2016), *Curcuma longa* (60.7%) *(*WAGHMARE et al.,2011), *Azadirachta indica* (55.6%)*, Camellia sinensis* (54.5%) *(*BALAPPANAVAR et al., 2013) e *Schinus terebinthifolius* (63.4%) (LINS et al., 2013). Esses resultados parecem ser devido ao efeito anti-inflamatório exercido pelas ervas que promovem ação inibitória da síntese de prostaglandinas, as quais desempenham papel importante nos eventos inflamatórios (SILVA et al.,2012).

Quanto ao desfecho halitose, a erva *Sesamum indicum* moutrou-se superior a CHX 0,2% para halitose auto-reportada, e com semelhante efeito no método organoléptico (ASOKAN et al., 2011). Já as ervas *G. mangostana, Cymbopogan citrates* e *Camellia sinensis* mostraram-se com efeito superior às soluções placebo no método halímeter (RASSAMEEMASMAUNG et al. 2007 e 2013) e organoléptico (SATTHANAKUL et al. 2015). Entretanto, estes resultados devem ser cuidadosamente avaliados pois os métodos de diagnóstico avaliados (auto-reportado e com Halímiter) não são tão adequados quanto o organoléptico, o qual é considerado padrão-ouro de diagnóstico para halitose (ROSENBERG et al., 1995).

Algumas limitações estão presentes nos estudos incluídos nesta dissertação. Considerações metodológicas com risco moderado de viés para a maioria deles. Além disso, poucos estudos (BHAT, 2014; BARATAKKE et al., 2017; GRAWISH et al., 2016; BALAPPANAVAR et al., 2013) relataram os efeitos colaterais das ervas, que é um aspecto importante a ser considerado para a indicação clínica segura desses produtos.

A maioria dos estudos incluídos nesta dissertação, independentemente dos desfechos avaliados, apresentou duração de curta a intermediária (sete dias a três meses). Apenas Grossman et al. (1989) realizaram uma análise por seis meses. Não há uma regulamentação quanto ao período de acompanhamento mínimo necessário para estudos que avaliem produtos com efeito na halitose (MORITA; WANG, 2001). Entretanto, a Associação Dental Americana (American Dental Association - ADA, 2008) e a Agência Nacional de Vigilância Sanitária (ANVISA,1999) estipulam um período mínimo de seis meses para a avaliação de eficácia e segurança dos enxaguatórios bucais com propostas anti-biofilme e anti-gengivite. Assim, há necessidade de estudos com maiores tempos de acompanhamento para avaliar a eficácia e segurança dos enxaguatórios a base de produtos ervais.

Além das limitações das revisões sistemáticas, já citadas anteriormente, a falta de estudos provenientes de literatura cinza foi uma das pricipais limitações presentes nessa dissertação, assim como a considerável heterogeneidade metodológica e clínica dos estudos primários.

Frente a essa análise, destacamos que a indicação de produtos ervais precisa ser alicerçada com evidências que verifiquem eficácia, segurança e efeitos adversos. Nossas sugestões para estudos futuros incluem: (1) estender o tempo de acompanhamento para 6 meses (2) analisar a dosagem ideal do produto erval e seu tempo de ação na cavidade bucal (3) adicionar análise de índice de manchas e efeitos adversos (4) acidionar e/ou controlar a remoção de saburra lingual nos estudos com desfecho halitose.

6 CONCLUSÃO

Esta dissertação demonstrou que produtos fitoterápicos como adjuvantes da higiene oral mostraram resultados superiores às soluções placebo e quando comparados a CHX as ervas *Camelia sinensis, Azadirachta indica, Anacardium occidentale Linn, Curcuma longa e Sesamun Indicum* apresentaram os melhores resultados nos desfechos biofilme dental, inflamação gengival e halitose. Entretanto, há muitos vieses na maioria dos estudos, o que compromete a certeza dessas conclusões. Assim, é necessário que novos ensaios clínicos randomizados sejam realizados, com melhor qualidade metodológica, maior tempo de duração e com a

inclusão de possíveis efeitos adversos como desfecho a ser também avaliado, antes que esses produtos possam ser recomendados.

REFERÊNCIAS

ADDY, M.; PRAYITNO, S.; TAYLOR, L.; CADOGAN, S. An in vitro study of the role of dietary factors in the aetiology of tooth staining associated with the use of chlorhexidine. **J Periodontal Res.**, v.14, p.403-410. 1979.

AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA (ANVISA). Resolução – RDC n.17 de 24 de fevereiro de 2000. **Diário Oficial da União.,** Brasília, DF, 2000. Dispõe sobre o registro de medicamentos fitoterápicos. Fev. 2000

AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA (ANVISA). Resolução – RDC. nº 335, de 22 de julho de 1999. **Diário Oficial da União.,** Brasília, DF, 1999. Dispõe sobre controle sanitário de produtos de higiene pessoal, cosméticos e perfumes. Jul. 1999.

ALBANDAR, J. M.; KINGMAN, A. Gingival recession, gingival bleeding, and dental calculus in Adults 30 years of age and older in the United States, 1988 - 1994. **J of Periodontology,** v. 70, n. 1, p. 1988–94, 1999.

AMBROSIO, S. R. et al. Antimicrobialactivity of kaurane diterpenes against oral pathogens. **Zeitschrift für Naturforschung. Section C: Biosciences**, Tubingen, v. 63, n. 5-6, p. 326-30, May-Jun. 2008.

AMERICAN DENTAL ASSOCIATION (ADA). Acceptance Program Guidelines: Chemotherapeutic Products for Control of Gingivitis. Council on Scientific Affairs, Available at: http://www.ada.org/en/science-research/ada-seal-of-acceptance/how-to-earn-the-ada-seal/guidelines-for-product-acceptance.

ASOKAN, S.; EMMADI, P.; SIVAKUMAR, N.; KUMAR, R.S.; RAGHURAMAN, R. Effect of oil pulling on halitosis and microorganisms causing halitosis: A randomized controlled pilot trial. **J Indian Soc Pedod Prev Dent** . v. 29(2) p.90. 2011.

AYERS, K. M.; & COLQUHOUN, A. N. Halitosis: causes, diagnosis, and treatment. **The New Zealand Dent J**, v.*94*(418), p.156-60.1998.

BALAPPANAVAR, A. Y.; SARDANA, V.; MALKEET, S. Comparison of the effectiveness of 0.5% tea, 2% neem and 0.2% chlorhexidine mouthwashes on oral health: A randomized control trial. **Indi J Dent Res**. V. 24, p. 26-34. 2013.

BARATA, L. E. S.; QUEIROZ, S. R. R. Contribuição efetiva ou potencial do PADCT para o aproveitamento econômico sustentável da biodiversidade. Campinas**: Ministério da Saúde e Tecnologia**, 1995.

BARATAKKE, S.; et al. Efficacy of triphala extract and chlorhexidine mouth rinse against plaque accumulation and gingival inflammation among female undergraduates: A randomized controlled trial. **Ind J of Dent Res**, v.*28*(1), p.49. 2017.

BATTINO, M. et al. In vitro antioxidant activities of mouthrinses and their components. **J Clin Periodontol**, Copenhagen, v. 29, n. 5, p. 462- 467, May. 2002.

BHAT, N.; et al. The antiplaque effect of herbal mouthwash in comparison to chlorhexidine in human gingival disease: A randomized placebo controlled clinical trial. **J of Comp and Int Med,** v.*11*(2), p.129-137. 2014.

BLOM, T.; SLOT, D.E; QUIRYNEN, M.; VAN DER WEIJDEN, G.A. The effect of mouthrinses on oral malodor: a systematic review. **Int J Dent Hyg**. v. 10, p.209-22, 2012.

BOLLEN, C. M.; BEIKLER, T. Halitosis: the multidisciplinary approach. **Int J Oral Sci** v.4, p. 55- 63, 2012.

BUCHAUL, R. B. Histórico da fitoterapia. **Série Cadernos de Fitoterapia.** Campinas: Ministério da Saúde e Tecnologia, 2001.

COHEN, J. P.; PAQUETTE, C.; CAIRNS, C. Switching prescription drug to over the counter. ***BMJ* 330,** p. 39-40, 2005.

CUNHA, L. C. S. et al. Antibacterial activity of triterpene acids and semi-synthetic derivatives against oral pathogens. **Zeitschrift für Naturforschung. Section C: Biosciences**, Tubingen, v. 62 c, p. 668-672, feb. 2007.

DE GEEST, S.; LALEMAN, I.; TEUGHELS, W.; DEKEYSER, C.; QUIRYNEN, M. Periodontal diseases as a source of halitosis: a review of the evidence and treatment approaches for dentists and dental hygienists. **Periodontology 2000** 71, p. 213 -227, 2016.

DELANGHE, G.; GHYSELEN, J.; VAN STEENBERGHE, D.; FEENSTRA L. Multidisciplinary breath-odour clinic. **Lancet** 350: 187 ,1997.

DEUTSCHER, H.C.D.; DERMAN, S.H.M.; BARBE, G.; SEEMANN, R.; NOACK, M.J. The effect of professional tooth cleaning or non-surgical periodontal therapy on oral halitosis in patients with periodontal diseases. A systematic review. **Int J Dent Hygiene**. p. 01-12, 2017.

DHINGRA, K.; VANDANA, K. L. Effectiveness of Azadirachta indica (neem) mouthrinse in plaque and gingivitis control: a systematic review. **Int J Dental Hygiene**, v.*15*(1), p. 04-15, 2017.

DI STASI, L.C. Plantas medicinais: verdades e mentiras: o que os usuários e os profissionais de saúde precisam saber. São Paulo: **UNESP**, 2007.

EISENBERG, D. M.; et al. Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. **JAMA**,v.280**,** p.1569-1575. 1998.

FUNIERU, C.; KLINGER, A.; BĂICUȘ, C.; FUNIERU, E.; DUMITRIU, H.T.; DUMITRIU, A. Epidemiology of gingivitis in schoolchildren in Bucharest, Romania: a cross-sectional study. **J Periodontal Res.** v.*52*, p.225-232. 2017.

FIGUEIREDO, E. et al. **Determinação da diluição inibitória máxima de um enxaguatório bucal à base de própolis e extratos vegetais**. In: REUNIÃO CIENTÍFICA DA SOCIEDADE BRASILEIRA DE PESQUISAS ODONTOLÓGICAS. São Paulo, 16., p.106, 1999.

FRANCISCO, K.S.F. Fitoterapia: Uma opção para o tratamento odontológico. **Revista Saúde**, v. 4, n.1, p. 18-24, 2010.

GOLDBERG, S.; KOZLOVSKY, A.; GORDON, D.; GELERNTER, I.; SINTOV, A.; ROSENBERG, M. Cadaverine as a putative component of oral malodor. **J Dent Res**, v. 73, n. 6, p. 1168-1172,1994.

GOMES, C.B., CAVALCANTE, D., FILHO, J.G., DA COSTA, F., DA SILVA PEREIRA, S. Clinical effect of a mouthwash containing Anacardium occidentale Linn. on plaque and gingivitis control: A randomized controlled trial. **Indian J Dent Res**, v.27(4), p.364. 2016.

GRANJEIRO, J. M. et al. O cloreto de cetilpiridínio e a placa bacteriana: uma revisão. **Rev A Paulista de C D**, São Paulo, v. 47, n. 2, p. 1019-1022, mar./abr. 1993.

GRAWISH, M.E., ANEES, M.M. Short-term effects of Verbena officinalis Linn decoction on patients suffering from chronic generalized gingivitis : Double-blind randomized controlled multicenter clinical trial. **Quintessence international Periodontology**. v.47(6), p.491-9. 2016.

GROSSMAN, E.; et al. Comparison of Antibacterial Mouthrinses : and Gingivitis Dental Plaque**. J Periodontol**. p. 435-40. 1989.

GUINNESS WORLD RECORDS. Gum disease. In: Guinness World Records. New York: Mint Publishers, Incorporated. p. 175. 2001.

GUNSOLLEY, J. C. Clincal efficacy of antimicrobial mouthrinses**. J of Dentistry** 38, p. 06-10, 2010.

HARRINGTON, P.; SHEPHERD, M. D. Analysis of the movement of prescription drugs to over-the-counter status. **J Manag Care Pharm,** p.499-508; 2002.

HEMAISWARYA, S; KRUTHIVENTI, A. K; DOBLE, M. Synergism between natural products and antibiotics against infectious diseases. **J Phyto and Phyto**, Stuttgart, v. 15, n. 8, p. 639-652, 2008.

HUGOSON, A.; SJODIN, B.; NORDERYD, O. Trends over 30 years, 1973-2003, in the prevalence and severity of periodontal disease. **J Clin Periodontol** *V.*35, p. 405-414, 2008.

KAPOOR, U.; SHARMA, G.; JUNEJA, M.; NAGPAL, A. Halitosis: Current concepts on etiology, diagnosis and management. **Eur J Dent**, v.10:, p.292-300. 2016.

KASSEBAUM, N.J; et al. Global burden of severe tooth loss: a systematic review and meta-analysis. **J Dent Res**, 93: 20S– 28S. 2014.

KATSURA, H. et al. In Vitro Antimicrobial Activties of Bakuchiol agoinst oral microorganisms. **Ant Ag and Chemo**, Washington, v.45, n.11, p. 3009-3013, Nov. 2001.

KOTHIWALE, S., PATWARDHAN, V., GANDHI, M., SOHONI, R., KUMAR, A. A comparative study of antiplaque and antigingivitis effects of herbal mouthrinse containing tea tree oil, clove, and basil with commercially available essential oil mouthrinse. **J Indian Soc Periodontol**, v.18(3), p.316. 2014.

KOZEL, C. **Saúde e cura pelas plantas e outros recursos naturais**. 13. ed. São Paulo: Casa Editora Firmamento, 1997.

LANG, N. P.; BRECX, M. Chlorhexidine digluconate – an agent for chemical plaque control and prevention of gingival inflammation **J Periodontal Res,** v. 21, p.74-89, 2006.

LI, X, C.; CAI, L.; WU, C. D. Antimicrobial compounds from Ceanothus americanus against oral pathogens**. Phytochemistry**, v.46, n.1, p.97-102, Sept. 1997.

LIU, X. N.; et al. Oral malodor -related parameters in the Chinese general population. **J Clin Periodontol,** v.33, p.31 -36. 2006.

LÖE, H.; RINDOM, S. C.; GLAVIND, L.; KARRING, T. Two years oral use of chlorhexidine in man**. J Periodontal Res**. v.11, p. 135-144. 1976.

LÖE, H., THEILADE, E. & JENSEN, S. B. Experimental Gingivitis in Man. **J Periodontol** *v.*36**,** p.177-187. doi:10.1902/jop.1965.36.3.177. 1965.

LOESCHE, W. J.; KAZOR, C. Microbiology and treatment of halitosis. **Periodontology 2000**, v.28, p. 256-279, 2002.

MADHUSHANKARI, G. S.; YAMUNADEVI A, S.; MOHAN K. K.; BASANDI, P. S. Halitosis – An overview: Part-I – Classification, etiology, and pathophysiology of halitosis. **J of Phar& Bio Sc**, v.7, p.339-343, 2015.

MANIPAL, S.; HUSSAIN, S.; WADGAVE, U.; DURAISWAMY, P.; & RAVI, K. The mouthwash war - Chlorhexidine vs. herbal mouth rinses: A meta-analysis. **J Clin Diag Res,** v.10, n.5, p.81-83, 2016.

MORAN, J. M. Home-use oral hygiene products: mouthrinses**. Periodontol 2000**, v. 48, p. 42-53, 2008.

MOREIRA, C. H. et al. Analyses of patients consumption profile of oral rinse solutions. Convention Center Exhibit Hall C, **Perio Res - Therapy**, v. 82, San Diego, 2002.

MOREIRA, A. C. A. et al. Avaliação in vitro da atividade antimicrobiana de antissépticos bucais. **Rev de C Méd e Biol**, Salvador, v. 8, n. 2, p. 153-161, 2009.

MARINHO, B. V. S.; ARAÚJO; A. C. S. O uso dos enxaguatórios bucais sobre a gengivite e o biofilme dental. **Int J of Dent**, Cairo, v. 6, n. 4, p. 124- 131. 2007.

MORITA, M.; WANG, H. L. Association between oral malodor and adult periodontitis: a review. **J Clin Periodontol**, *v. 28*(9), p.813–819. Journal Article, Review. 2001.

MURRAY, J.J.; VERNAZZA, C.R.; HOLMES, R.D. Forty years of national surveys: an overview of children's dental health from 1973–2013. **Br Dent J.** v. *219*, p. 281–285. 2015.

MARSH, P.D. Dental plaque: biological significance of a biofilm and community life-style. **J Clin Periodontol**, v.32, p.7 -15, 2005.

OUTHOUSE, T. L.**;** FEDOROWICZ, Z.**;** KEENAN J. V.**;** AL-ALAWI, R. A. Cochrane systematic review finds tongue scrapers have short-term efficacy in controlling halitosis. **Gen Dent.** Sep-Oct; 54(5): 352-9; 360, 367-8; quiz 360. 2006.

PORTO, T. S. et al. Pimarane-type diterpenes: antimicrobial activity against oral pathogens. **Molecules,** Basel, v. 14, n. 1, p.191-199, Jan. 2009.

PRIYA, M.; et al. Efficacy of chlorhexidine and green tea mouthwashes in the management of dental plaque-induced gingivitis: A comparative clinical study. **Contemp Clin Dent**, v. 6(4), p. 505. 2015.

RAMBERG, P.; AXELSSON, P.; LINDHE J. Plaque formation at healthy and inflamed gingival sites in Young individuals. **J Clin Periodontol**, v. 22, p. 85-88, 1995.

RASSAMEEMASMAUNG, S. et al. Effects of herbal mouthwash containing the pericarp extract of Garcinia mangostana L on halitosis, plaque and papillary bleeding index. **J of the Int A of Periodontol**, v.9, n. 1, p.19-25, 2007.

RASSAMEEMASMAUNG , S.; PHUSUDSAWANG, P.; SANGALUNGKARN, V. Effect of Green Tea Mouthwash on Oral Malodor. **Hindawi Publishing Corporation**. p.6, 2013.

ROSENBERG, M. et al. Self-estimation of oral malodor. **Dent Res**. v.74(9), p.1577-82, Sep 1995.

ROSENBERG, M.; KULKARNI, G. V.; BOSY, A.; McCULLOCH, C. A. G. Reproducibility and sensitivity of oral malodor measurements with a portable sulphide monitor. **J Dent Res**, v. 70, n. 11, p. 1436-1440, 1991.

RÖSING, C.K.; MALTZ, M.; OPPERMANN, R.V. Controle químico do biofilme supragengival. **Cadernos da ABO PREV V**, p. 1-8, maio 2005.

SANZ, M.; VAN WINKELHOFF, A. J.; Working Group 1 of Seventh European Workshop on, P. Periodontal infections: understanding the complexity--consensus of the Seventh European Workshop on Periodontology. **J Clin Periodontol***,* v.38, Suppl 11, p. 3-6. 2011.

SATTHANAKUL, P. et al. Antimicrobial effect of lemongrass oil against oral malodour micro-organisms and the pilot study of safety and efficacy of lemongrass mouthrinse on oral malodour. **Journal of Applied Microbiology**, v.118 n.1, p. 11–17, 2015.

SCHATZLE, M. et al. Clinical course of chronic periodontitis. I. Role of gingivitis. **J Clin Periodontol,** v.30, p. 887-901, 2003.

SHEEN, E.; OWENS, J.; ADDY, M. The effect of toothpaste on the propensity of chlorhexidine and cetylpiridinium chloride to produce staining in vitro: a possible predictor of inactivation. **Journal of Clinical Periodontology**, Malden, v. 28, n. 1, p. 46, Jan 2001.

SILVA, M.I.; DE MELO, C.T.; VASCONCELOS, L.F.; DE CARVALHO, A.M.; SOUSA, F.C. Bioactivity and potential therapeutic benefits of some medicinal plants from the Caatinga (semi-arid) vegetation of Northeast Brazil: A review of the literature**. Braz J Pharmacogn**, v. 22, p. 193-207, 2012.

SILVA, M. F. et al. Estimated prevalence of halitosis: a systematic review and meta-regression analysis. **Clinical Oral Investigations**. https://doi.org/10.1007/s00784-017-2164-5, 2017.

SUSIN, C.; OPPERMANN, R.V.; HAUGEJORDEN, O.; ALBANDAR, J.M. Tooth loss and associated risk indicators in an adult urban population from south Brazil. **Acta Odontol Scand**; v. 63, p. 85-93, 2005.

SUSIN, C. et al. Periodontal attachment loss in an urban population of Brazilian adults: effect of demographic, behavioral, and environmental risk indicators. **Journal of Periodontology**, v. 75, n. 7, p. 1033-41, Jul 2004.

TROMBELLI, L.; SCAPOLI, C.; TATAKIS, D.N.; GRASSI, L. Modulation of clinical expression of plaque-induced gingivitis: effects of personality traits, social support and stress. **J Clin Periodontol**, v.32, p. 1143-1150, 2005.

VAN DER SLEEN, M. I.; SLOT, D. E.; VAN TRIJFFEL, E.; WINKEL, E. G.; VAN DER WEIJDEN, G. A. Effectiveness of mechanical tongue cleaning on breath odour and tongue coating: a systematic review. **Int J Dent Hyg**. v.8, p. 258-68. 2010.

VAN DER WEIJDEN, G. A.; HIOE, K. P. A systematic review of the effectiveness of self-performed mechanical plaque removal in adults with gingivitis using a manual toothbrush. **J Clin Periodontol,** v.32 Suppl 6**,** p. 214-228, 2005.

VAN LEEUWEN, M. P. C.; SLOT, D. E.; VAN DER WEIJDEN. G. A. Essential Oils Compared to Chlorhexidine With Respect to Plaque and Parameters of Gingival Inflammation: A Systematic Review. **J Periodontology**, v.*82*, n. 2, p.174-194, 2011.

VAN STRYDONCK, D. A. C.; SLOT, D. E.; VAN DER VELDEN, U.; VAN DER WEIJDEN, F. Effect of a chlorhexidine mouthrinse on plaque, gingival inflammation and staining in gingivitis patients: A systematic review. **J Clin Periodontology**, v. *39*, n.11, p. 1042-1055, 2012.

VARONI, E.M.; LODI, G.; SARDELLA, A.; CARRASSI, A.; IRITI, M. Plant polyphenols and oral health: oldphytochemicals for new fields. **Curr Med Chem**, v.19, n. 11, p.1706-1720, 2012.

WAGHMARE, P.F.; CHAUDHARI, A.U.; KARHADKAR, V.M.; JAMKHANDE, A.S. Comparative evaluation of turmeric and chlorhexidine gluconate mouthwash in prevention of plaque formation and gingivitis: A clinical and microbiological study. **J Contemp Dent Pract**, v.12, n.4, p. 221–4, 2011.

WILLERSHAUSEN, B.; GRUBER, I.; HAMM, G. Índice de placa e sangramentos gengivais: a influência de ingredientes herbários. **Rev. Ass. Paulista de Cirurgiões Dentista**, São Paulo, v.48, n.3, p. 1335-1340, maio/jun 1994.

WILSON, R.M.; DANISHEFSKY, S.J. Small molecule natural products in the discovery of therapeutic agents: the synthesis connection. **J Org Chem**, v. 71, n.22, Oct 2006.

WORLD HEALTH ORGANIZATION – WHO. WHA 30.49 **Promotion and development of training and research in traditional medicine**, In: 30th. World Healtyh Assembly; p. 02-19, Geneva. May 1977.

WORLD HEALTH ORGANIZATION-WHO. **Drug policies and management: Medicinal Plants**, WHO Document WHA 31. 33. Geneva: WHO, 1978.

YAEGAKI, K.; COIL, J. M. Genuine halitosis, pseudo -halitosis, and halitophobia: classification, diagnosis, and treatment. **Compendium of Continuing Education in Dentistry**, v.21, p. 880-886, 2000.

YUNES, R. A.; PEDROSA, R. C.; YURI, V. C. Fármacos e fitoterápicos: a necessidade do desenvolvimento da indústria de fitoterápicos e fitofármacos no Brasil. **Química Nova**, São Paulo, v. 24, n. 1, p. 147-152, jan./fev. 2001.

ANEXO A – NORMAS PARA PUBLICAÇÃO NO PERIÓDICO *JOURNAL OF CLINICAL PERIODONTOLOGY*

Author Guidelines

Content of Author Guidelines: 1. General, 2. Ethical Guidelines, 3. Manuscript Submission Procedure, 4. Manuscript Types Accepted, 5. Manuscript Format and Structure, 6. After Acceptance

Relevant Document: Sample Manuscript

Useful Websites: Submission Site, Articles published in *Journal of Clinical Periodontology*, Author Services, Wiley-Blackwell's Ethical Guidelines, Guidelines for Figures

The journal to which you are submitting your manuscript employs a plagiarism detection system. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

1. GENERAL

Journal of Clinical Periodontology publishes original contributions of high scientific merit in the fields of periodontology and implant dentistry. Its scope encompasses the physiology and pathology of the periodontium, the tissue integration of dental implants, the biology and the modulation of periodontal and alveolar bone healing and regeneration, diagnosis, epidemiology, prevention and therapy of periodontal disease, the clinical aspects of tooth replacement with dental implants, and the comprehensive rehabilitation of the periodontal patient. Review articles by experts on new developments in basic and applied periodontal science and associated dental disciplines, advances in periodontal or implant techniques and procedures, and case reports which illustrate important new information are also welcome.

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in Journal of Clinical Periodontology. Authors are encouraged to visit Wiley-Blackwell's Author Services for further information on the preparation and submission of articles and figures.

2. ETHICAL GUIDELINES

Journal of Clinical Periodontology adheres to the below ethical guidelines for publication and research.

2.1. Authorship and Acknowledgements

Authors submitting a paper do so on the understanding that the manuscript have been read and approved by all authors and that all authors agree to the submission of the manuscript to the Journal.

Journal of Clinical Periodontology adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisition of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under Acknowledgements.

Please note that it is a requirement to include email addresses for all co-authors at submission. If any of the email-addresses supplied are incorrect the corresponding author will be contacted by the journal administrator.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited.

2.2. Ethical Approvals

Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above-mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of

Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

2.3 Clinical Trials

Clinical trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A CONSORT checklist should also be included in the submission material.

Journal of Clinical Periodontology encourages authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following free, public clinical trials registries: www.clinicaltrials.gov, http://clinicaltrials.ifpma.org/clinicaltrials/, http://isrctn.org/. The clinical trial registration number and name of the trial register will then be published with the paper.

2.4 DNA Sequences and Crystallographic Structure Determinations

Papers reporting protein or DNA sequences and crystallographic structure determinations will not be accepted without a GenBank or Brookhaven accession number, respectively. Other supporting data sets must be made available on the publication date from the authors directly.

2.5 Conflict of Interest and Source of Funding

Journal of Clinical Periodontology requires that all authors (both the corresponding author and co-authors) disclose any potential sources of conflict of interest. Any interest or relationship, financial or otherwise that might be perceived as influencing an author’s objectivity is considered a potential source of conflict of interest. These must be disclosed when directly relevant or indirectly related to the work that the authors describe in their manuscript. Potential sources of conflict of interest include but are not limited to patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. If authors are unsure whether a past or present affiliation or relationship should be disclosed in the

manuscript, please contact the editorial office at cpeedoffice@wiley.com. The existence of a conflict of interest does not preclude publication in this journal.

The above policies are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals produced by the International Committee of Medical Journal Editors (http://www.icmje.org/). It is the responsibility of the corresponding author to have all authors of a manuscript fill out a conflict of interest disclosure form, and to upload all forms together with the manuscript on submission. The disclosure statement should be included under Acknowledgements. Please find the form below:

Conflict of Interest Disclosure Form

2.6 Appeal of Decision

Under exception circumstances, authors may appeal the editorial decision. Authors who wish to appeal the decision on their submitted paper may do so by e-mailing the editorial office at cpeedoffice@wiley.com with a detailed explanation for why they find reasons to appeal the decision.

Please note that all revisions and resubmissions of papers should also include a separate rebuttal and a tracked changes document to assist in peer review.

2.7 Permissions

If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publishers.

3. MANUSCRIPT SUBMISSION PROCEDURE

Manuscripts should be submitted electronically via the online submission site http://mc.manuscriptcentral.com/jcpe. The use of an online submission and peer review site enables immediate distribution of manuscripts and consequentially speeds up the review process. It also allows authors to track the status of their own manuscripts. Complete instructions for submitting a paper is available on the submission site. Further assistance can be obtained from the Senior Editorial Office Assistant, Kim Harris, at cpeedoffice@wiley.com.

Please note that all revisions and resubmissions of papers should also include a separate rebuttal and a tracked changes document to assist in peer review.

3.1. Manuscript Files Accepted

Main manuscripts should be uploaded as Word (.doc) or Rich Text Format (.rft) files (not write-protected). The text file must contain the entire manuscript including title page, abstract, clinical reference, main text, references, acknowledgement, statement of source of funding and any potential conflict of interest, tables, and figure legends, but no embedded figures. In the text, please reference any figures as for instance 'Figure 1', 'Figure 2' etc. to match the tag name you choose for the individual figure files uploaded.

Figure files should be uploaded separately to the main text. GIF, JPEG, PICT or Bitmap files are acceptable for submission, but only high-resolution TIF or EPS files are suitable for printing.

Manuscripts should be formatted as described in the Author Guidelines below.

Please ensure that ALL items (figures and tables) are cited in the main text.

3.2. Blinded Review

All manuscripts submitted to Journal of Clinical Periodontology will be reviewed by two or more experts in the field. Papers that do not conform to the general aims and scope of the journal will, however, be returned immediately without review. Journal of Clinical Periodontology uses single blinded review. The names of the reviewers will thus not be disclosed to the author submitting a paper.

3.3. Suggest a Reviewer

Journal of Clinical Periodontology attempts to keep the review process as short as possible to enable rapid publication of new scientific data. In order to facilitate this process, please suggest the name and current email address of one potential international reviewer whom you consider capable of reviewing your manuscript. In addition to your choice the editor will choose one or two reviewers as well.

3.4. Suspension of Submission Mid-way in the Submission Process

You may suspend a submission at any phase before clicking the 'Submit' button and save it to submit later. The manuscript can then be located under 'Unsubmitted Manuscripts' and you can click on 'Continue Submission' to continue your submission when you choose to.

3.5. E-mail Confirmation of Submission

After submission you will receive an e-mail to confirm receipt of your manuscript. If you do not receive the confirmation e-mail after 24 hours, please check your e-mail address carefully in the system. If the e-mail address is correct please contact your IT

department. The error may be caused by some sort of spam filtering on your e-mail server. Also, the e-mails should be received if the IT department adds our e-mail server (uranus.scholarone.com) to their whitelist.

3.6 Resubmissions

If your manuscript was given the decision of reject and resubmit, you might choose to submit an amended version of your manuscript. This should be submitted as a new submission following the guidelines above under 3.2. In addition you should upload comments to the previous review as “supplementary files for review”.

4. MANUSCRIPT TYPES ACCEPTED

Journal of Clinical Periodontology publishes original research articles, reviews, clinical innovation reports and case reports. The latter will be published only if they provide new fundamental knowledge and if they use language understandable to the clinician. It is expected that any manuscript submitted represents unpublished original research.

Original Research Articles must describe significant and original experimental observations and provide sufficient detail so that the observations can be critically evaluated and, if necessary, repeated. Original articles will be published under the heading of clinical periodontology, implant dentistry or pre-clinical sciences and must conform to the highest international standards in the field.

Clinical Innovation Reports are suited to describe significant improvements in clinical practice such as the report of a novel surgical technique, a breakthrough in technology or practical approaches to recognized clinical challenges. They should conform to the highest scientific and clinical practice standards.

Case Reports illustrating unusual and clinically relevant observations are acceptable but their merit needs to provide high priority for publication in the Journal. On rare occasions, completed cases displaying non-obvious solutions to significant clinical challenges will be considered.

Reviews are selected for their broad general interest; all are refereed by experts in the field who are asked to comment on issues such as timeliness, general interest and balanced treatment of controversies, as well as on scientific accuracy. Reviews should take a broad view of the field rather than merely summarizing the authors´ own previous work, so extensive citation of the authors´ own publications is discouraged. The use of state-of-the-art evidence-based systematic approaches is

expected. Reviews are frequently commissioned by the editors and, as such, authors are encouraged to submit a proposal to the Journal. Review proposals should include a full-page summary of the proposed contents with key references.

5. MANUSCRIPT FORMAT AND STRUCTURE

5.1. Format

Language: The language of publication is English. Authors for whom English is a second language may choose to have their manuscript professionally edited before submission to improve the English. It is preferred that manuscript is professionally edited. Please refer to English Language Editing Services offered by Wiley at http://wileyeditingservices.com/en/.

Japanese authors can also find a list of local English improvement services at http://www.wiley.co.jp/journals/editcontribute.html. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication.

Abbreviations, Symbols and Nomenclature: Journal of Clinical Periodontology adheres to the conventions outlined in Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors. Abbreviations should be kept to a minimum, particularly those that are not standard. Non-standard abbreviations must be used three or more times and written out completely in the text when first used.

5.2. Structure

All articles submitted to Journal of Clinical Periodontology should include:

Title Page

Conflict of Interest and Source of Funding

Clinical Relevance

Abstract

Introduction

Materials and Methods

Results

Discussion

References

Tables (where appropriate)

Figure Legends (where appropriate)

Figures (where appropriate and uploaded as separate files)

All manuscripts should emphasize clarity and brevity. Authors should pay special attention to the presentation of their findings so that they may be communicated clearly. Technical jargon should be avoided as much as possible and be clearly explained where its use is unavoidable.

Title Page: The title must be concise and contain no more than 100 characters including spaces. The title page should include a running title of no more than 40 characters; 5-10 key words, complete names of institutions for each author, and the name, address, telephone number, fax number and e-mail address for the corresponding author.

Conflict of Interest and Source of Funding: Authors are required to disclose all sources of institutional, private and corporate financial support for their study. Suppliers of materials (for free or at a discount from current rates) should be named in the source of funding and their location (town, state/county, country) included. Other suppliers will be identified in the text. If no funding has been available other than that of the author's institution, this should be specified upon submission. Authors are also required to disclose any potential conflict of interest. These include financial interests (for example patent, ownership, stock ownership, consultancies, speaker's fee,) or provision of study materials by their manufacturer for free or at a discount from current rates. Author's conflict of interest (or information specifying the absence of conflicts of interest) and the sources of funding for the research will be published under a separate heading entitled "Conflict of Interest and Source of Funding Statement".

See Editor-in-Chief Maurizio Tonetti's Editorial on Conflict of Interest and Source of Funding and www.icmje.org/#conflicts for generally accepted definitions.

Abstract: is limited to 200 words in length and should not contain abbreviations or references. The abstract should be organized according to the content of the paper.

For Original Research Articles the abstract should be organized with aim, materials and methods, results and conclusions.

For clinical trials, it is encouraged that the abstract finish with the clinical trial registration number on a free public database such as clinicaltrials.gov.

Clinical Relevance: This section is aimed at giving clinicians a reading light to put the present research in perspective. It should be no more than 100 words and should not be a repetition of the abstract. It should provide a clear and concise explanation

of the rationale for the study, of what was known before and of how the present results advance knowledge of this field. If appropriate, it may also contain suggestions for clinical practice.

It should be structured with the following headings: scientific rationale for study, principal findings, and practical implications.

Authors should pay particular attention to this text as it will be published in a highlighted box within their manuscript; ideally, reading this section should leave clinicians wishing to learn more about the topic and encourage them to read the full article.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited.

5.3. Original Research Articles

These must describe significant and original experimental observations and provide sufficient detail so that the observations can be critically evaluated and, if necessary, repeated. Original articles will be published under the heading of clinical periodontology, implant dentistry or pre-clinical sciences and must conform to the highest international standards in the field.

The word limit for original research articles is 3500 words, and up to 7 items (figures and tables) may be included. Additional items can be included as supplementary files online (please see 5.9 below).

Main Text of Original Research Articles should be organized with

Introduction,

Materials and Methods,

Results and Discussion.

References (Harvard, see section 5.7)

The background and hypotheses underlying the study, as well as its main conclusions, should be clearly explained. Please see Sample Manuscript.

Introduction: should be focused, outlining the historical or logical origins of the study and not summarize the results; exhaustive literature reviews are not appropriate. It should close with the explicit statement of the specific aims of the investigation.

Material and Methods: must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced. As a condition of publication, authors are required to make materials and

methods used freely available to academic researchers for their own use. This includes antibodies and the constructs used to make transgenic animals, although not the animals themselves.

(a) Clinical trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A CONSORT checklist should also be included in the submission material. If your study is a randomized clinical trial, you will need to fill in all sections of the CONSORT Checklist. If your study is not a randomized trial, not all sections of the checklist might apply to your manuscript, in which case you simply fill in N/A.

Journal of Clinical Periodontology encourages authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following free, public clinical trials registries: www.clinicaltrials.gov, http://clinicaltrials.ifpma.org/clinicaltrials/. The clinical trial registration number and name of the trial register will then be published with the paper.

(b) Statistical Analysis: As papers frequently provide insufficient detail as to the performed statistical analyses, please describe with adequate detail. For clinical trials intention to treat analyses are encouraged (the reasons for choosing other types of analysis should be highlighted in the submission letter and clarified in the manuscript).

(c) DNA Sequences and Crystallographic Structure Determinations: Papers reporting protein or DNA sequences and crystallographic structure determinations will not be accepted without a Genbank or Brookhaven accession number, respectively. Other supporting data sets must be made available on the publication date from the authors directly.

(d) Experimental Subjects: Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

Results: should present the observations with minimal reference to earlier literature or to possible interpretations.

Discussion: may usefully start with a brief summary of the major findings, but repetition of parts of the abstract or of the results section should be avoided. The discussion section should end with a brief conclusion and a comment on the potential clinical relevance of the findings. Statements and interpretation of the data should be appropriately supported by original references.

The discussion may usefully be structured with the following points in mind (modified from the proposal by Richard Horton (2002), The Hidden Research Paper, The Journal of the American Medical Association, 287, 2775-2778). Not all points will apply to all studies and its use is optional, but we believe it will improve the discussion section to keep these points in mind.

Summary of key finding

* Primary outcome measure(s)
* Secondary outcome measure(s)
* Results as they relate to a prior hypothesis

Strengths and Limitations of the Study

* Study Question
* Study Design
* Data Collection
* Analysis
* Interpretation
* Possible effects of bias on outcomes

Interpretation and Implications in the Context of the Totality of Evidence

* Is there a systematic review to refer to?
* If not, could one be reasonably done here and now?
* What this study adds to the available evidence
* Effects on patient care and health policy
* Possible mechanisms

Controversies Raised by This Study Future Research Directions

* For this particular research collaboration
* Underlying mechanisms
* Clinical research

5.4. Clinical Innovation Reports

These are suited to describe significant improvements in clinical practice such as the report of a novel surgical technique, a breakthrough in technology or practical approaches to recognized clinical challenges. They should conform to the highest scientific and clinical practice standards.

The word limit for clinical innovation reports is 3000 words, and up to 12 items (figures and tables) may be included. Additional items can be included as supplementary files online (please see 5.9 below).

The main text of Clinical Innovation Reports should be organized with

Introduction,

Clinical Innovation Report,

Discussion and Conclusion

References (see section 5.7)

5.5. Case Reports

Case reports illustrating unusual and clinically relevant observations are acceptable but their merit needs to provide high priority for publication in the Journal. On rare occasions, completed cases displaying non-obvious solutions to significant clinical challenges will be considered.

The main text of Case Reports should be organized with

Introduction,

Case report,

Discussion and Conclusion

References (see section 5.7)

5.6. Reviews

Reviews are selected for their broad general interest; all are refereed by experts in the field who are asked to comment on issues such as timeliness, general interest and balanced treatment of controversies, as well as on scientific accuracy. Reviews should take a broad view of the field rather than merely summarizing the authors´ own previous work, so extensive citation of the authors´ own publications is discouraged. The use of state-of-the-art evidence-based systematic approaches is expected. Reviews are frequently commissioned by the editors and, as such, authors are encouraged to submit a proposal to the Journal. Review proposals should include a full-page summary of the proposed contents with key references.

The word limit for reviews is 4000 words.

The main text of Reviews should be organized with

Introduction,

Review of Current Literature,

Discussion and Conclusion

References (see section 5.7)

5.7. References

It is the policy of the Journal to encourage reference to the original papers rather than to literature reviews. Authors should therefore keep citations of reviews to the absolute minimum.

References should be prepared according to the Publication Manual of the American Psychological Association (6th edition). This means in text citations should follow the author-date method whereby the author's last name and the year of publication for the source should appear in the text, for example, (Jones, 1998). The complete reference list should appear alphabetically by name at the end of the paper.

A sample of the most common entries in reference lists appears below. Please note that a DOI should be provided for all references where available. For more information about APA referencing style, please refer to the APA FAQ. Please note that for journal articles, issue numbers are not included unless each issue in the volume begins with page one.

Journal article

Beers, S. R. , & De Bellis, M. D. (2002). Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. The American Journal of Psychiatry, 159, 483–486. doi:10.1176/appi.ajp.159.3.483

Book

Bradley-Johnson, S. (1994). Psychoeducational assessment of students who are visually impaired or blind: Infancy through high school (2nd ed.). Austin, TX: Pro-ed.

Chapter in an Edited Book

Borstrøm, I., & Elbro, C. (1997). Prevention of dyslexia in kindergarten: Effects of phoneme awareness training with children of dyslexic parents. In C. Hulme & M. Snowling (Eds.), Dyslexia: Biology, cognition and intervention (pp. 235–253). London: Whurr.

Internet Document

Norton, R. (2006, November 4). How to train a cat to operate a light switch [Video file]. Retrieved from http://www.youtube.com/watch?v=Vja83KLQXZs

Please note that all unpublished papers (submitted or in press) included in the reference list should be provided in a digital version at submission. The unpublished paper should be uploaded as a supplementary file for review.

5.8. Tables, Figures and Figure Legends

Tables: should be double-spaced with no vertical rulings, with a single bold ruling beneath the column titles. Units of measurements must be included in the column title.

Figures: All figures should be planned to fit within either 1 column width (8.0 cm), 1.5 column widths (13.0 cm) or 2 column widths (17.0 cm) and must be suitable for photocopy reproduction from the printed version of the manuscript. Lettering on figures should be in a clear, sans serif typeface (e.g. Helvetica); if possible, the same typeface should be used for all figures in a paper. After reduction for publication, upper-case text and numbers should be at least 1.5-2.0 mm high (10 point Helvetica). After reduction symbols should be at least 2.0-3.0 mm high (10 point). All half-tone photographs should be submitted at final reproduction size. In general, multi-part figures should be arranged as they would appear in the final version. Each copy should be marked with the figure number and the corresponding author's name. Reduction to the scale that will be used on the page is not necessary, but any special

requirements (such as the separation distance of stereo pairs) should be clearly specified.

Unnecessary figures and parts (panels) of figures should be avoided: data presented in small tables or histograms, for instance, can generally be stated briefly in the text instead. Figures should not contain more than one panel unless the parts are logically connected; each panel of a multipart figure should be sized so that the whole figure can be reduced by the same amount and reproduced on the printed page at the smallest size at which essential details are visible.

Figures should be on a white background, and should avoid excessive boxing, unnecessary color, shading and/or decorative effects (e.g. 3-dimensional skyscraper histograms) and highly pixelated computer drawings. The vertical axis of histograms should not be truncated to exaggerate small differences. The line spacing should be wide enough to remain clear on reduction to the minimum acceptable printed size. Figures divided into parts should be labelled with a lower-case, boldface, roman letter, a, b, and so on, in the same type size as used elsewhere in the figure. Lettering in figures should be in lower-case type, with the first letter capitalized. Units should have a single space between the number and the unit and follow SI nomenclature or the nomenclature common to a particular field. Thousands should be separated by thin spaces (1 000). Unusual units or abbreviations should be spelled out in full or defined in the legend. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. In general, visual cues (on the figures themselves) are preferred to verbal explanations in the legend (e.g. broken line, open red triangles etc.)

Preparation of Electronic Figures for Publication

Although low quality images are adequate for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit EPS (lineart) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented programmes. Scans (TIFF only) should have a resolution of 300 dpi (halftone) or 600 to 1200 dpi (line drawings) in relation to the reproduction size (see below). EPS files should be saved with fonts embedded (and with a TIFF preview if possible). For scanned images, the scanning resolution (at final image size) should be as follows to

ensure good reproduction: lineart: >600 dpi; half-tones (including gel photographs): >300 dpi; figures containing both halftone and line images: >600 dpi.

Detailed information on our digital illustration standards can be found at http://authorservices.wiley.com/bauthor/illustration.asp.

Check your electronic artwork before submitting it: http://authorservices.wiley.com/bauthor/eachecklist.asp.

Guidelines for Cover Submission

If you would like to send suggestions for artwork related to your manuscript to be considered to appear on the cover of the journal, please follow these guidelines.

Permissions: If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publishers.

Figure Legends: should be a separate section of the manuscript and should begin with a brief title for the whole figure and continue with a short description of each panel and the symbols used; they should not contain any details of methods.

5.9. Supplementary Material

Supplementary material, such as data sets or additional figures or tables that will not be published in the print edition of the Journal but which will be viewable in the online edition, can be uploaded as 'Supporting information for review and online publication only'.

Please see http://authorservices.wiley.com/bauthor/suppmat.asp for further information on the submission of Supplementary Materials.

6. AFTER ACCEPTANCE

Upon acceptance of a paper for publication, the manuscript will be forwarded to the Production Editor who is responsible for the production of the journal.

6.1 Proof Corrections

The corresponding author will receive an email alert containing a link to a web site. A working email address must therefore be provided for the corresponding author. The proof can be downloaded as a PDF (portable document format) file from this site. Acrobat Reader will be required in order to read this file. This software can be downloaded (free of charge) from the following Web site: www.adobe.com/products/acrobat/readstep2.html . This will enable the file to be opened, read on screen, and printed out in order for any corrections to be added.

Further instructions will be sent with the proof. Hard copy proofs will be posted if no e-mail address is available; in your absence, please arrange for a colleague to access your e-mail to retrieve the proofs. Proofs must be returned to the Production Editor within three days of receipt. As changes to proofs are costly, we ask that you only correct typesetting errors. Excessive changes made by the author in the proofs, excluding typesetting errors, will be charged separately. Other than in exceptional circumstances, all illustrations are retained by the publisher. Please note that the author is responsible for all statements made in his work, including changes made by the copy editor.

6.2 Early View (Publication Prior to Print)

The Journal of Clinical Periodontology is covered by Wiley-Blackwell's Early View service. Early View articles are complete full-text articles published online in advance of their publication in a printed issue. Early View articles are complete and final. They have been fully reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after online publication. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the traditional way. They are therefore given a Digital Object Identifier (DOI), which allows the article to be cited and tracked before it is allocated to an issue. After print publication, the DOI remains valid and can continue to be used to cite and access the article.

6.3 Production Tracking

Online production tracking is available for your article once it is accepted by registering with Wiley-Blackwell's Author Services.

6.4 Accepted Articles

'Accepted Articles' have been accepted for publication and undergone full peer review but have not been through the copyediting, typesetting, pagination and proofreading process. Accepted Articles are published online a few days after final acceptance, appear in PDF format only (without the accompanying full-text HTML) and are given a Digital Object Identifier (DOI), which allows them to be cited and tracked. The DOI remains unique to a given article in perpetuity. More information about DOIs can be found online at http://www.doi.org/faq.html. Given that Accepted Articles are not considered to be final, please note that changes will be made to an article after

Accepted Article online publication, which may lead to differences between this version and the Version of Record. The Accepted Articles service has been designed to ensure the earliest possible circulation of research papers after acceptance. Given that copyright licensing is a condition of publication, a completed copyright form is required before a manuscript can be processed as an Accepted Article.

Accepted articles will be indexed by PubMed; therefore the submitting author must carefully check the names and affiliations of all authors provided in the cover page of the manuscript, as it will not be possible to alter these once a paper is made available online in Accepted Article format.

6.5 Video Abstracts

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