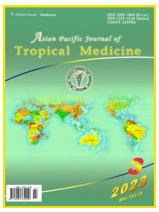

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# *Nigella sativa* oil as a treatment for gingivitis: A randomized active−control trial

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## ABSTRACT

**Objective:** To assess the clinical anti-inflammatory and antimicrobial efficacy of *Nigella sativa* oil compared with chlorhexidine in patients with gingivitis.

**Methods:** A double-blind, randomized clinical trial was conducted in patients having chronic generalized gingivitis. Patients were randomly assigned to receive *Nigella sativa* oil ($n$=18) or chlorhexidine ($n$=19). The following assessments were made on day 0 and day 15: plaque index, gingival index, gingival IL-6 and IL-18 levels were measured using ELISA, plaque colony-forming units, and alpha-hemolytic *Streptococcus* strains. Data were analyzed using parametric and non-parametric tests and Fisher's exact test.

**Results:** Both interventions reduced plaque index and gingival index scores ($P$<0.000 1). The *Nigella sativa* oil group was better at lowering IL-6 ($P$=0.007 6) than the chlorhexidine group ($P$=0.145), although there was no change in IL-18 levels ($P$>0.05). The post-intervention plaque index and gingival index scores and inflammatory cytokine levels between the two groups were not significantly different. Both interventions caused a significant reduction in the plaque colony-forming units ($P$<0.000 1), reducing pathogenic bacteria: *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus sanguinis*, and *Streptococcus parasanguinis* in the chlorhexidine group (50%) ($P$=0.103 1), and the *Nigella sativa* oil group (20%) ($P$=0.739 5).

**Conclusions:** *Nigella sativa* oil had anti-inflammatory and antibacterial activities, reducing biofilm formation and disrupting the colonization of pathogenic bacteria essential for the progression of periodontal disease. *Nigella sativa* oil could offer an alternative therapy for treating gingivitis and may prevent associated systemic diseases and improve overall health outcomes.

**KEYWORDS:** Chlorhexidine; Mouth rinse; Interleukin; Gingival index; Plaque index

## 1. Introduction

Oral diseases are an important public health problem worldwide. Dental caries and periodontal diseases afflict most of the world's population[1]. The most common type of gum disease is gingivitis, and dental plaque is one of the main etiologic factors initiating periodontal diseases[1]. The presence of microbial biofilm adjacent to the gingival tissues can lead to inflammation; hence the removal of the biofilm is needed to reverse the condition. If gingivitis is left untreated, it progresses to periodontal disease, which involves bone loss[1].

**Significance**

We sought to identify if a 14-day regimen of mouth rinsing with *Nigella sativa* oil compared with the gold standard chlorhexidine at reducing clinical parameters, local gingival inflammation, and bacterial load in patients with gingivitis. The results showed that *Nigella sativa* oil caused a reduction in gingival crevicular IL-6, gingival inflammation, plaque accumulation, and reduction of bacterial load. *Nigella sativa* was equally efficacious as chlorhexidine at reducing clinical parameters and thus may be prescribed to patients for gingivitis as an alternative to chemical mouthwashes. Furthermore, *Nigella sativa* oil may improve oral and general health and prevent periodontal-associated systemic diseases.

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Furthermore, with recent development in oral health and systemic disease research, it is established that periodontal disease and its associated bacteria, particularly *Porphyromonas (P.) gingivalis*, may be related to the progression of Alzheimer's and dementia[2-5] and periodontal disease is associated with a higher risk cardiovascular disease and incidents such as myocardial infarct and stroke[6]. Therefore, the health implications of maintaining oral health may be far-fetched and as such identifying safe, efficacious, and simple preventative and adjunctive treatment options for gingivitis is important since brushing alone is not always effective at reversing the condition. Although the prevalence of severe periodontal disease in the adult population is 20% worldwide[7], it is believed that the preceding condition, gingivitis is likely to be the most common infectious inflammatory condition worldwide. In 2014, the prevalence of gingivitis was reported to be 100% in the Saudi adult population (aged 18-40 years old)[8], and in South America, over 95% were reported to have gingivitis[9]. The etiology of gingivitis can differ from person to person.

The common form is plaque-induced gingivitis. In addition, nutritional gingivitis may occur due to a deficiency in vitamin C, or high glycemic index carbohydrates could also stimulate gingival inflammation by inducing pro-inflammatory cytokine production and increasing oxidative stress. In addition, a high ratio of omega 6 to omega 3 fats promotes the inflammatory response and may contribute to the progression of gingivitis by weakening the defense mechanisms at the gingival interface[10]. Hormonal gingivitis is typically induced by estrogen[11] and, as such, could occur at any time in a female menstrual cycle, depending on her individual hormone balance. However, it is widely known to occur in pregnant women, attributed to increased body fluid and blood flow, as well as the propensity for dilated blood vessels[10,12], rendering the gingival line defense system more tenuous to attack. Finally, drug-induced gingivitis or gingival growth may occur and is notable with some well-known and widely used drugs such as phenytoin anti-epileptic drug classes, a calcium channel blocker, and an older generation of oral contraceptives[11]. With many postulated mechanisms, one well-established process is by increasing epidermal derived growth factor (EGF) and platelet-derived growth factor (PDGF) orchestrated by the interaction of prostaglandins and interleukins such as interleukin-6 (IL-6) and those involved in angiogenesis resulting in fibroblast cell growth and collagen deposition[10,13]. It is important to note that even in non-plaque-induced gingivitis, plaque can play a precipitating role.

The incidence and progression rate of periodontal disease depends on the multifaceted interactions between periodontopathic bacteria and cells of the host immune system[14]. A complex microbiological bio flora exists in the oral cavity, and an imbalance may encourage the appearance of inflammatory processes leading to gingivitis and, subsequently, periodontitis[15]. An accumulation of supragingival plaque causes the most common form of gingivitis. The early colonizers are the alpha-hemolytic streptococci found in all individuals at all oral cavity sites and predominate in the saliva and the soft tissues of the oral cavity[16]. The late colonizers, including pathogenic high virulence Gram-negative bacteria, adhere and attach to the *Streptococcus* within the biofilm, switching on the body's immune responses and leading to periodontal disease[17]. *Streptococcus (S.) oralis*, *S. sanguinis*, *S. mitis*, and other viridans and other streptococci importantly make up a considerable part of the young plaque associated with gingival health leading to gingivitis[18].

The interactions between the bacteria and the body are mediated by cytokines and chemokines, which are produced mainly by resident and emigrant cells at the site of inflammation. Cells that produce cytokines include macrophages/monocytes, dendritic cells, lymphocytes, neutrophils, endothelial cells, and fibroblasts. Cytokines are central to the pathogenesis of many chronic inflammatory diseases, including periodontal disease[19]. IL-6 is a multifunctional cytokine that plays a vital role in the inflammatory response to infectious agents (especially gram-negative bacteria)[19]. IL-18 is a pro-inflammatory cytokine of the IL-1 superfamily upregulated in various chronic diseases, including periodontal disease[14].

Many patients cannot maintain adequate plaque control despite brushing and preventive procedures. Therefore, over-the-counter antimicrobial mouth rinses such as Listerine and chlorhexidine (CHX) are commonly used adjuncts to prevent gingivitis and periodontal diseases[1]. Some antibacterial agents such as chlorhexidine, fluorides, and various antibiotics have been reported to exhibit undesirable side effects such as nausea, vomiting, allergy, and tooth staining[20]. Furthermore, an increasing trend in herbal therapeutics drives the need to identify a natural product that may be useful for preventing periodontal disease. There are many reports of using common oils, such as coconut, for rinsing the mouth[21]. This method of oil pulling is known to facilitate the removal of bacteria mechanically. Using herbal oils with intrinsic antibacterial, anti-inflammatory, and anti-oxidative properties would be an added benefit.

*Nigella sativa* (NS) or black seeds are used widely in several countries as a flavoring agent in foods and to treat various systemic diseases such as asthma, bronchitis, and rheumatism. In Islamic belief, it is considered to be a cure for all diseases except death. NS has many pharmacologically active compounds; one of the most reported is thymoquinone having antioxidant, anti-inflammatory, and anti-microbial activity[22]. Thymoquinone has been extensively studied and is known to exert several therapeutic pharmacological effects, such as regulating blood lipid levels and blood sugar levels and controlling tumor growth and having protective effects on

multiple body organs[23]. In addition, NS and thymoquinone are known to be beneficial in treating oral conditions, thus promoting oral health[22]. NS was effective against multiple antibiotic-resistant bacteria, including *S. aureus*, *Pseudomonas aeruginosa* and the fungus *Candida albicans*[24]. Furthermore, NS was effective against the oral bacteria *S. mutans* and *S. sanguis*[25]. The cold-pressed NS oil is available in food stores for oral consumption, but it has a strong bitter taste; despite this, the NS oil is consumed orally in an undiluted form[26], and a previous study reported the cold-pressed oil was active against the periodontal pathogen *P. gingivalis* at a 10% concentration[27].

Therefore, it is important to identify natural, safer, more tolerable, and efficacious treatment options for preventing periodontitis, which may also prove to be beneficial for improving overall health. We decided to assess the impact of NS oil as a mouthwash in treating gingivitis, we hypothesized that NS oil would be comparable in efficacy to CHX in treating gingivitis. We designed the present clinical trial to answer the following research question: Does a 14-day regimen of mouth rinsing with NS oil compare with the gold standard CHX in reducing clinical parameters, local gingival inflammation, and bacterial load in patients with gingivitis? The primary outcomes were the clinical parameters: gingival and plaque index and levels of gingival crevicular IL-6 and IL-18, whereas the secondary outcomes were the overall bacterial load and assessment of alpha-hemolytic plaque-causing bacteria.

## 2. Materials and methods

### 2.1. Participant enrolment

A total of 40 systemically healthy participants, aged between 20 and 40 years with chronic generalized gingivitis, were recruited from the Dental Clinic at Princess Nourah bint Abdulrahman University, in accordance with the Code of Ethics of the World Medical Association Declaration of Helsinki 2008, with the following inclusion and exclusion criteria. The participants' oral diagnosis and history were reviewed and confirmed by a general dentist.

Inclusion criteria were as follows: (1) at least 20 natural teeth; (2) patients with moderate to severe gingivitis; (3) no tooth attachment loss. Exclusion criteria were as follows: (1) patients who have had periodontal treatment in the last 6 months; (2) acute oral infection; (3) oral habits-chewing tobacco/betel leaf; (4) cigarette smokers; (5) antibiotic therapy in the previous 3 months; (6) systemic diseases; (7) pregnant women; (8) lactating women; (9) current orthodontic treatment; (10) using an intra-oral artificial prosthesis; (11) using chemical agent mouthwash.

The sample size was calculated to be 23 having 80% power to detect a difference in means of 0.16, assuming a standard deviation of difference of 0.22, using a paired $t$-test with a 5% two-sided significance level. The mean values were based on the mean plaque index and standard deviations described by Amoian *et al*[28]. Previous studies conducted to assess the effectiveness of herbal products on periodontal disease also used a sample size of 15-20 participants, where a statistically significant difference in the parameters after the intervention was reported[29,30]. Each participant was provided with a study information sheet, and the clinical trial methodology was explained. Written informed consent was obtained before enrolling in the study.

### 2.2. Study design

A double-blind, randomized parallel clinical trial was conducted according to the 2008 Declaration of Helsinki and 2010 CONSORT (Consolidated Standards of Reporting Trials) guidelines. Ethical clearance was obtained from the Institutional Review Board at Princess Nourah bint Abdulrahman University (Registration number: 20-0261). The study has been registered at Clinicaltrials.gov (Identifier: NCT05069246).

Participants were blindly assigned into two equal groups using computer-generated random numbers by a person not directly involved in patient examination and data analysis; Group 1-NS ($n$=20) or Group 2-CHX ($n$=20). The interventions were either 10 mL 50% (5 mL NS + 5 mL water) NS oil (Al-Hussan Food Products Factory, Riyadh, Kingdom of Saudi Arabia) or 10 mL 0.2% CHX (Middle East Pharmaceutical Industries Ltd, Riyadh, Kingdom of Saudi Arabia). Participants were given standard oral hygiene instructions. In addition, they were given fluoridated toothpaste, and a soft-bristled toothbrush, the modified bass method of brushing technique was taught, and they were instructed to brush twice daily. The participants used either one of the interventions, rinsing for 3 min twice a day; in the morning and at night, 30 min after tooth brushing. The participants were provided with a 24-hour contact number to report any concerns, adverse reactions, or for further information.

Clinical measurements and samples were taken on day 0 and day 15. Care providers performed oral prophylaxis on day 0 (baseline) after data collection to standardize the plaque scores. Participants were given either NS or CHX by an investigator not directly involved in the clinical examination and sample collection. An investigator blind to the randomization and allocation of interventions to the participants performed interleukin and microbial assessments. The study design is summarized in Figure 1.

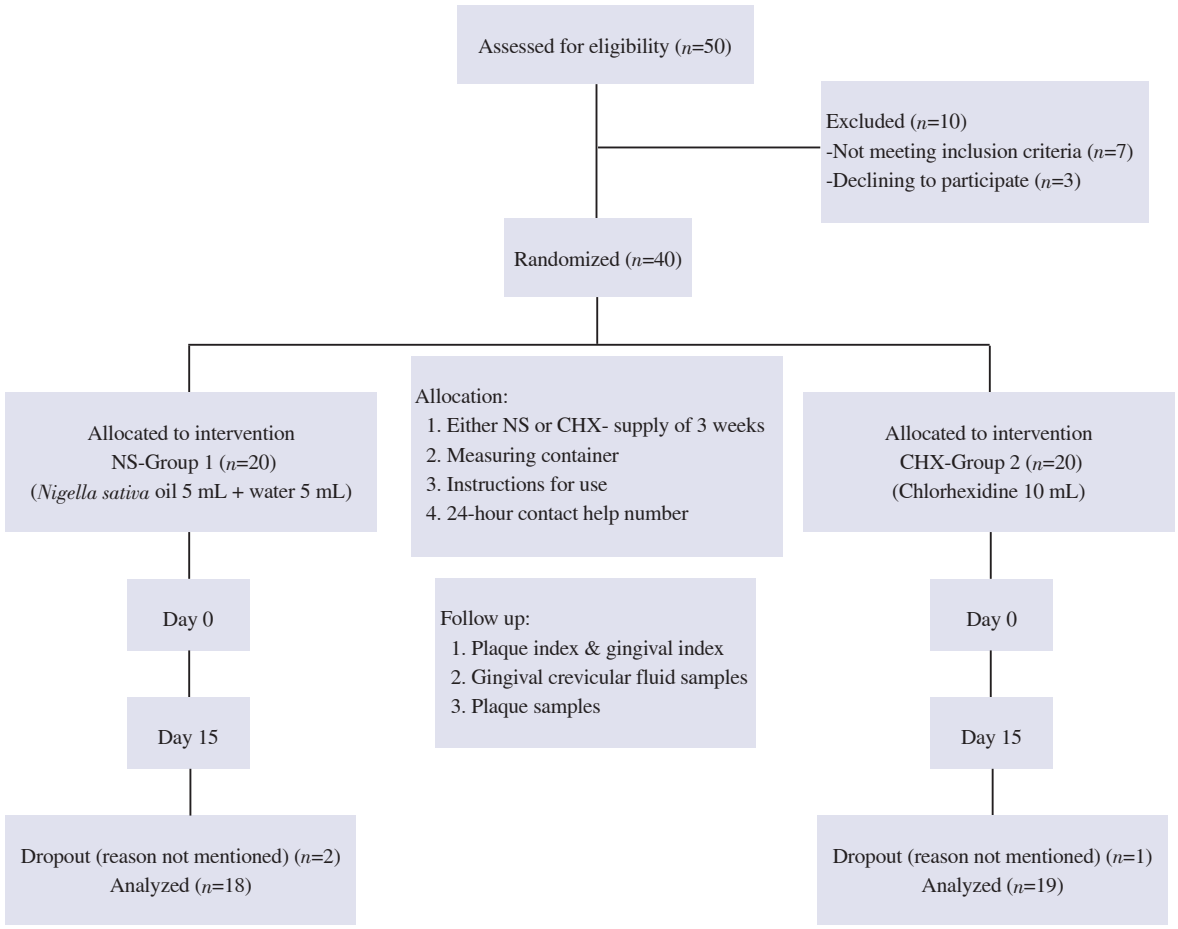


**Figure 1.** Flowchart of the study design.

### *2.3. Measurement of plaque index (PI) and gingival index (GI)*

Before the study, two dental examiners were calibrated to measure PI and GI by an expert in measuring indices to reduce inter-examiner variability. The PI and GI were measured from the buccal surface from teeth # 16, 12, 24, 32, and 44, as reported previously[31].

### *2.4. Gingival crevicular fluid (GCF) sample collection*

GCF samples were collected from the upper central incisor by absorption technique, as reported previously, using paper points (Mata Biomed Co Ltd, Korea)[32]. GCF samples were stored at -80 ℃ until needed for analysis.

### *2.5. Plaque sample collection and culture identification*

Plaque samples were collected from the buccal surface of the first upper molar using a sterile excavator. Plaque samples were cultured overnight at 30 ℃ in 1 mL of sterile Luria-Bertani Broth (Sigma-Aldrich, Miller, USA) with shaking and subsequently cultured on blood agar plates (MilliporeSigma, USA). Colony-forming units were quantified from overnight plaque cultures, and bacteria were directly identified using the VITEK® 2 ID system as reported earlier[33].

### *2.6. Measurement of IL-6 and IL-18*

Enzyme-linked immunosorbent assay (ELISA) was used according to the manufacturer's instructions to measure the interleukin levels using a high sensitivity human IL-6 ELISA kit (Abcam, UK) and a human IL-18 ELISA kit (Abcam, UK).

### *2.7. Statistical analysis*

Data were analyzed using GraphPad PRISM (San Deigo, CA, USA) non-parametric signed-rank tests and parametric *t*-tests were used, as well as Fisher's exact test for contingency analysis. *P* values were calculated, and a *P*-value below 0.05 was deemed as a significant difference.

## 3. Results

Forty patients were enrolled in a 14-day clinical trial, 20 in each group. Baseline measurements were taken on day 0, and final

measurements were taken on day 15. Three participants dropped out: 2 from Group 1 (NS) and 1 from Group 2 (CHX). Therefore, 18 participants were analyzed in the NS group, and 19 were analyzed in the CHX group. The dropouts gave no reasons for not continuing in the trial. In addition, during the term of the study, none of the participants reported any adverse effects.

### *3.1. Clinical evaluation and levels of gingival IL–6 and IL–18*

NS and CHX caused a significant reduction in the GI and PI scores ($P$<0.000 1). The percentage of GI reduction for the NS group was 83.90% ($n$=18), while in the CHX group, a slightly greater percentage reduction was achieved: 89.07% ($n$=19). The percentage reduction in the PI for the NS group was 74.47%, while in the CHX group, it was 84.31%. In addition, there was no significant reduction in GCF IL-18 levels with either NS ($P$=0.284) or CHX ($P$=0.418). On the other hand, a significant drop in IL-6 levels occurred with NS intervention ($P$=0.007 0) but not with CHX ($P$=0.083 1). Interestingly, there was no significant difference between NS and CHX groups in day 0 baseline and day 15 post-intervention GI and PI scores and in levels of GCF IL-6 and IL-18. Results are summarized in Table 1 and Figure 2 and 3.

**Table 1.** Participant demographic data and clinical parameters in NS and CHX intervention study groups at day 0.

| Variables | NS [$n$=18, mean±SD (95% *CI*)] | CHX [$n$=19, mean±SD (95% *CI*)] |
|---|---|---|
| Age, years | 25.6 ± 2.6 | 24.5 ± 3.0 |
| GI | 1.74 ± 0.22 (1.63-1.85) | 1.83 ± 0.23 (1.72-1.94) |
| PI | 1.41 ± 0.34 (1.24-1.62) | 1.53 ± 0.37 (1.35-1.71) |
| IL-6 (pg/mL) | 1.57 ± 2.72 (0.30-2.84) | 1.30 ± 1.82 (0.45-2.16) |
| IL-18 (pg/mL) | 797 ± 412 (604-990) | 893 ± 378 (716-1 070) |
| CFU | 300* (300-300) | 300* (300-300) |

GI, PI, and CFU data were analyzed using a paired $t$-test and an unpaired $t$-test. GCF IL-6 and IL-18 levels were analyzed using the Kruskal-Walis Dunn's Multiple comparisons test. No significant difference was found in the baseline information (day 0). All participants were females. *Maximum cut-off reading ns (no significant difference, $P$>0.999); GCF: gingival crevicular fluid, NS: *Nigella sativa*, CHX: chlorhexidine, GI: gingival index , PI: plaque index, CFU: colony forming units.

The power of the study was calculated to be 87% based on the mean reduction and SD of the gingival index for each group (Table 1), calculated using a group sample size of 18 and an effect size of 0.773 with a 5% two-sided significance level.

### *3.2. Bacterial load and biofilm quality*

Figure 4 showed that the number of colony forming units (CFU) exceeded the maximum reading cutoff of 300 in pre-intervention NS and CHX samples. The CFU was significantly reduced with both interventions ($P$<0.000 1). However, CHX was considerably better at

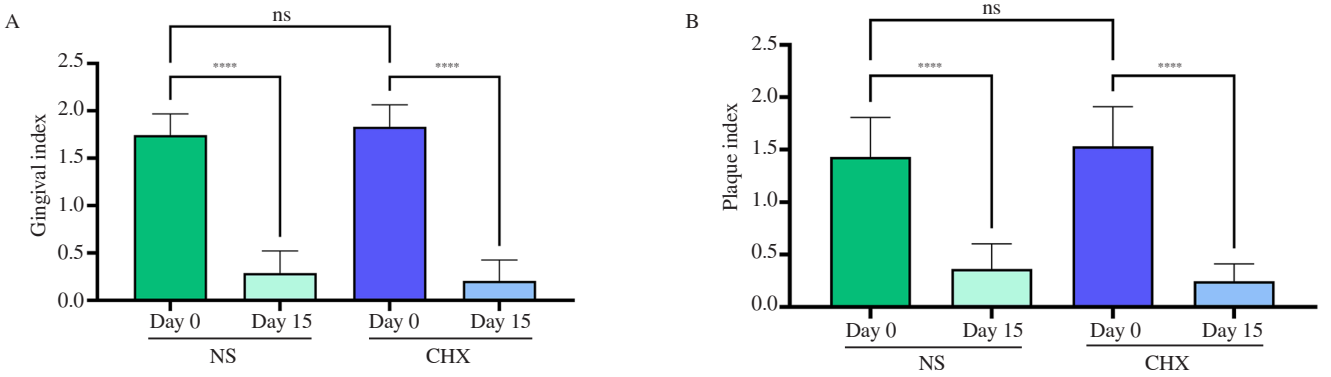


**Figure 2.** Gingival index scores (A) and plaque index scores (B) in patients before (day 0) and after intervention (day 15) with *Nigella sativa* (NS) or chlorhexidine (CHX). Data were analyzed using a paired $t$-test (within groups) and an unpaired t-test (between groups). ****$P$<0.000 1, ns: no significant difference.

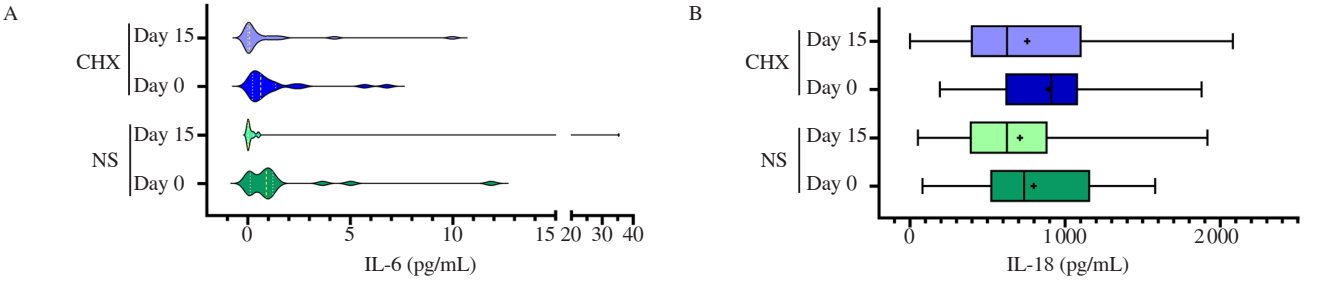


**Figure 3.** IL-6 (A) and IL-18 (B) levels (pg/mL) in gingivitis patients before (day 0) and after intervention (day 15) with *Nigella sativa* (NS) or chlorhexidine (CHX). Data were analyzed using the Kruskal-Walis Dunn's multiple comparisons test. Median and quartiles are indicated in violin plot (A) and box and whisker plot (B), mean (+).

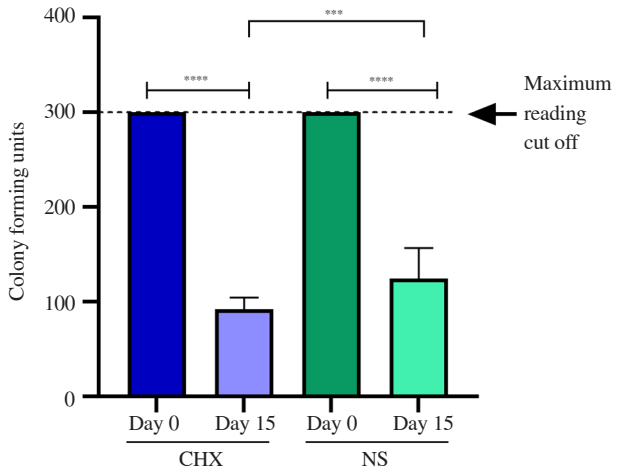


**Figure 4.** Bacterial load was measured as colony forming units (CFU) in supragingival plaque samples taken from patients before (day 0) and after intervention (day 15) with *Nigella sativa* (NS) or chlorhexidine (CHX). Data were analyzed using a paired *t*-test (within groups) and an unpaired *t*-test (between groups). $^{***}P<0.001$, $^{****}P<0.000\,1$.

lowering the CFU than NS ($P=0.000\,2$).

In the NS group ($n=18$), 10 patients had one or more pathogenic strains of bacteria at baseline, and the number was reduced to 8 after the intervention. Therefore, the NS intervention caused a 20% reduction in the number of patients with pathogenic bacteria ($P=0.739\,5$). In the CHX group ($n=19$), 12 patients had one or more pathogenic bacteria at baseline, and the number was reduced to 6 at the end of the CHX intervention. Thus, CHX intervention caused a 50% reduction in the number of patients with pathogenic bacteria ($P=0.103\,1$).

## 4. Discussion

Increasing awareness about chemical agents, drug risks, and adverse effects fuels the demand for natural and readily available oral healthcare products. One of the most gained assets derived from dental practice is the potential to prevent periodontal disease by a simple but effective measure through daily dental plaque control. Although CHX has strong clinical and microbiological effects, it is reported to have many undesirable side effects[20]. On the other hand, NS may be a safer natural candidate for maintaining oral health, fostered by its antimicrobial and anti-inflammatory effects[22]. Previous studies have compared the effect of CHX and essential oils on plaque index and gingival index[28].

In some cases, clinical trials assessing CHX were conducted for 2 weeks or less[28] and others for longer[34]. We chose a 14-day study term based on the standard prescribed duration of use for CHX. To our knowledge, this is the first study to investigate the effect of NS oil *in vivo* on patients with gingivitis while assessing gingival health, microbial load, and changes in the biofilm.

The NS oil used was an edible cold-pressed oil that preserves the phytochemicals to a greater degree; it is reported to have higher levels of thymoquinone and other volatile and non-volatile active components, including fatty acids and bioactive lipid components[35,36]. Thymoquinone is a highly lipophilic molecule[23]; it penetrates well into biological membranes, a property that would facilitate its absorption from the oral mucosa. The oral mucosa is an explored site of drug delivery and considering the poor oral bioavailability of thymoquinone[23], efficient absorption from the highly vascularized site of the oral cavity is an appealing prospect. This would allow greater bioavailability of thymoquinone due to the direct entry into the circulatory system, avoiding first-pass metabolism in the gastrointestinal tract and liver[37], thus rinsing the mouth with NS oil may not only impact oral health and indirectly systemic conditions associated with oral health but also directly positively impact the general health of an individual dictated by the known therapeutic properties of NS and thymoquinone. Oil pulling is an ancient ayurvedic therapy for maintaining oral hygiene[25]. In the current study, a 1:1 ratio of NS oil to water was used to facilitate the mouth rinsing process and dilute the strong bitter taste.

Both NS and CHX mouth rinse significantly reduced gingival and plaque scores, although CHX showed a greater efficacy. One study showed clinical improvement of gingivitis in diabetic mice treated with NS seed oil[38]. In a clinical split-mouth study with 24 patients having moderate-to-severe gingivitis, the efficacy of ethanolic extract of NS was tested and proved effective in treating moderate to severe gingivitis[39]. Furthermore, intra-crevicular application of 0.2% thymoquinone gel in patients with periodontitis showed potential as an adjunct to scaling and root planing treating chronic periodontitis[40]. Interestingly, the NS group showed a remarkable reduction of the gingival and plaque index scores, thus highlighting its ability to interfere with the bacterial host interactions, most likely influencing the gingival tissue *via* its anti-inflammatory effects. Furthermore, NS oil appeared to be equally efficacious and comparable to the gold standard CHX at reducing clinical parameters.

Although bacteria are the primary etiologic factors in periodontal disease, the patient's host response determines disease susceptibility and severity. In the current study, the evaluation of IL-6 and IL-18 in the GCF was considered a useful indicator of periodontal disease and response to treatment. We found no significant difference in the efficacy of the interventions NS and CHX at reducing GCF inflammatory cytokines IL-6 or IL-18. However, there was a significant reduction in IL-6 within the NS group comparing baseline to day 15, but no effect on the IL-18 levels for both interventions. An

important feature to note is the significantly higher levels of the IL-18 than the IL-6 levels found in the gingiva of the patients, which may be a sign of the degree of inflammation occurring in mild to moderate gingivitis. Other reports showed that IL-6 levels were higher in chronic inflammatory diseases such as rheumatoid arthritis and periodontitis and were related to an increased risk of periodontitis and appeared to be integral in promoting periodontitis[41]. Therefore, it may be expected for IL-6 to be lower than the IL-18 in the case of gingivitis. CHX has previously been reported to have cytotoxic effects on different cell types such as gingival fibroblasts, epithelial cells, and neutrophils[42,43] and displayed genotoxic effects[44]. CHX induced pro-inflammatory cytokines (IL-6, IL-18, and others) in odontoblast cell lines[45] and caused oral mucosal irritation[46]; thus, CHX appears to have no intrinsic anti-inflammatory effect, and its clinical anti-inflammatory effect seems to be *via* its strong effect as an antimicrobial agent.

In contrast, NS has cytoprotective and intrinsic anti-inflammatory effects[24]. Other studies showed that scaling and root planing effectively reduced GCF levels of IL-18 from inflamed periodontal sites in chronic periodontitis patients[47]. Therefore, scaling and root planing may be required for effective reductions of IL-18. The present study was limited to 14 days, and a more extended treatment period may be needed for NS oil to observe its anti-inflammatory effects fully.

Intervention with NS oil and CHX caused a significant reduction in the CFU, demonstrating NS's effectiveness in significantly reducing bacterial load, although CHX was better. It is well-documented that CHX mouthwash is associated with a significant change in the salivary microbiome because of its high acidity and low availability of nitrates[48]. Changing the microbial environment could be the main reason for the shifting in microbial density associated with CHX. Therefore, a significant reduction in CFU was expected with the use of CHX. Furthermore, NS oil had antimicrobial activity against microbes isolated from periodontal patients (buccal surface[15]. Other studies proved NS oil to be effective against pathogenic periodontal bacteria, *P. gingivalis*[27], and bacteria that make up the early biofilm[25]. Its active constituent, thymoquinone, was highly effective against oral biofilm formation[49]. NS and CHX interventions provided incomplete removal of pathogenic species: *S. parasanguinis, S. sanguinis, S. mitis, S. oralis,* and a host of non-pathogenic species initially present in the supra-gingival plaque of the patients. The presence of *S. parasanguinis* has always been associated with dental caries[50].

In contrast, *S. mitis* and *S. oralis* could indicate early-stage decay[48] and periodontal diseases[51]. After treatment with NS, a 20% reduction in the number of patients with pathogenic bacteria was observed, and the pathogenic species were limited to *S. mitis* and *S. parasanguinis*. In the CHX group, there was a 50% reduction in the number of patients with the pathogenic bacteria, and the detected species after treatment were only *S. sanguinis* and *S. parasanguinis*. Although not significant, our findings show the effect of the different interventions on the qualitative alterations of the biofilm, which can be expected due to the innate differences in the properties of these interventions.

Together, our study identifies no difference in clinical efficacy between NS and CHX in primary assessed outcomes of GI and PI measurements and levels of IL-6 and IL-18. Although NS was efficacious at reducing bacterial load, CHX was significantly better. Thus, NS oil may be an alternative therapeutic option in treating mild to moderate gingivitis.

There were limitations to the study. A more extended observation period or an increase in NS active ingredient concentration could be required for more anti-bacterial therapeutic efficacy. Traditional oil pulling utilizes undiluted oils; however, since 10% NS oil was effective as an anti-microbial against *P. gingivalis*[27], we proposed to use a 50% oil concentration to dilute the bitter taste and reduce non-compliance by our participants. An 87% power of the study validates the number of participants used in this clinical trial, but a larger number would improve the reliability of the data overall. Since convenience sampling was conducted at the dental clinic, the entire sample in the current study consisted of females due to the clinic having a greater number of female registered patients.

Furthermore, it is well known that hormonal fluctuations can influence gingival inflammation. Variations in gingival inflammation and GCF IL-6 levels occur in women at different times of the menstrual cycle[52]. Higher levels of progesterone and estradiol which occur during pregnancy may cause greater gingival inflammation[53]. Similarly, women taking hormonal contraceptives may experience greater gingival inflammation[54]. In the current study, we excluded pregnant females from our sample. However, other cyclical hormonal changes in our study participants may have impacted the findings. Therefore, we shed light on the effect of NS oil mouthwash in this susceptive and diverse population.

Thymoquinone has also been reported to inhibit the production of pro-inflammatory cytokines such as NF-B, inhibit angiogenic processes[55], reduce pro-inflammatory prostaglandin levels[56], and reduce EGF levels[57]. In addition, NS contains important antioxidants and phytoestrogens[58,59]. Furthermore, the cold-pressed NS oil was shown to potentiate omega-3 anti-inflammatory effects in obesity-induced oxidative stress and inflammation[60]. Therefore, the NS cold-pressed oil may be useful in nutritional gingivitis aggravated by a high carbohydrate diet. The effect of NS on hormonally induced gingivitis would be an interesting area for further study, and considering its role in regulating angiogenesis, inflammatory cytokines, eicosanoids, and growth factors, it may be a worthy candidate for managing drug-induced gingivitis.

The study also relied on the individual participant to mix a precise ratio of NS oil to water and then rinse for 3 min. Although not reported, it could be that inaccuracies in the ratio of NS to water may have occurred, which would lead to skewed results. The study also relied on self-reporting to identify the level of compliance. In the future, we want to limit the degree of bias by having a more controlled clinical trial. No adverse effects were reported from any of the participants in the study, and thus, our findings indicate that 50% NS oil in a water mix may be safe for short-term use for up to 14 days.

In conclusion, using natural herbal products may be a safer therapeutic alternative to standard chemical mouthwashes for maintaining oral hygiene and preventing periodontal diseases. NS would appeal to patients who wish to avoid chemical and artificial mouthwashes. We found NS clinically comparable to the gold standard CHX with efficacy at reducing gingival inflammation and influencing the bacterial biome, controlling biofilm formation by possibly breaking down the interaction between the host immune system and pathogenic biofilm bacteria, inevitably preventing periodontal diseases. Furthermore, this would positively impact oral health and improve health outcomes in patients at risk of periodontal disease-associated conditions. In addition, NS may be useful in the management of nutritional gingivitis and drug-induced gingivitis and furthermore may influence hormonally induced gingivitis, which necessitates studies assessing the impact of NS oil on these conditions. The use of NS oil as an oral mucosal mouth rinse is attractive, identifying the need for research on drug delivery through this approach identifying its impact in the treatment of systemic diseases and maintenance of general health. As such, clinicians should consider NS oil rinse as an alternative to chlorhexidine.

## Conflict of interest statement

All authors have confirmed that there are no conflicts of interest.

## Ethics approval and consent to participate

The authors certify that the study was performed in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was granted ethical approval by the Institutional Review Board at Princess Nourah bint Abdulrahman University (Registration number: 20-0261). Written informed consent was obtained from each participant before enrolling in the study.

## Consent for publication

Patients signed informed consent regarding publishing data generated from this study.

## Availability of data and materials

The study has been registered at Clinicaltrials.gov (Identifier: NCT05069246). The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Authors' contributions

IR and MGO contributed to the study conception and design. Material preparation and data collection were performed by IR, AM AlAnoud Alghazlan, and AlAnoud Alwably. Data analysis was performed by IR and MH. The first draft of the manuscript was written by IR, MAA, and MGO. All authors read and approved the final manuscript.

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## Publisher's note