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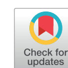
Original Article

Formulation of an *Origanum vulgare* based dental gel with antimicrobial activity



Karakoz Zh. Badekova, MSc [a,*], Gayane A. Atazhanova, DSc [a], Tomas Kacergius, DSc [b], Saule B. Akhmetova, PhD [c] and Marlen K. Smagulov, PhD [d]

[a] *Department of Pharmaceutical Sciences and Chemistry, School of Pharmacy, Karaganda Medical University, Kazakhstan*
[b] *Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Lithuania*
[c] *Department of Biomedicine, Karganda Medical University, Kazakhstan*
[d] *Department of Biology, Academician E.A. Buketov Karaganda University, Kazakhstan*

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الملخص

أهداف البحث: تهدف هذه الدراسة إلى صياغة هلام الأسنان أورجانوم فالجاري الجديد المضاد للتسوس مع فاعلية عالية في مضادات الميكروبات.

طرق البحث: تم استخراج الزيت العطري أورجانوم فالجاري باستخدام التقطير المائي؛ واستخراج الإيثانول، واللوني للغاز/قياس الطيف الكتلي، وطرق الكروماتوغرافيا السائلة عالية الأداء. وتم تحديد النشاط المضاد للميكروبات للهلام المنتج بزيت الأوريجانو والمستخلص بطريقة الانتشار القرصي. وتم اختبار فعالية الزيت العطري أورجانوم فالجاري في المختبر من أجل الفلم الحيوي للعقدية الطافرة باستخدام التحليل اللوني.

النتائج: منع زيت أورجانوم فالجاري العطري من نمو الفلم الحيوي للعقدية الطافرة بنسبة ٩٨٪ مقارنة ببكتيريا التحكم غير المكشوفة. وتمت صياغة خمس عينات من الجل المضاد للتسوس باستخدام زيت الأوريجانو الأساسي والمستخلص. بناء على نتائج الدراسة الميكروبيولوجية، أظهرت عينات الهلام الأولى والثالثة نشاطا عاليا لمضادات الميكروبات ضد السلالات البكتيرية موجبة الجرام من المكورات العنقودية الذهبية والعصوية الرقيقة وفطريات المبيضة البيضاء ونشاطا معتدلا مضادا للميكروبات ضد السلالات سالبة الجرام من الإشريكية القولونية والزائفة الزائفة.

الاستنتاجات: بناء على نتائج هذه الدراسة، يمكن اعتبار عينة الهلام الثالثة بمثابة مادة هلامية مضادة للتسوس نظرا لارتفاع نشاطها المضاد للميكروبات. تتميز هذه العينة بخصائص حسية جيدة مقارنة بالعينات الأخرى، وتحمل نشاطا مضادا للميكروبات مرتفعا نسبيا وتحمي من الأغشية الحيوية المسببة للسرطان من العقدية الطافرة.

الكلمات المفتاحية: العوامل المضادة للبكتيريا؛ الأغشية الحيوية؛ الجل؛ الأوريجانوم؛ المستخلصات النباتية؛ المكورات العنقودية الذهبية

Abstract

Objectives: This study aims to formulate a new *Origanum vulgare* anti-caries dental gel with high antimicrobial activity.

Methods: *O. vulgare* essential oil was extracted using hydro-distillation, ethanol extraction, gas chromatography/mass spectrometry, and high-performance liquid chromatography methods. Antimicrobial activity of the produced gels with oregano oil and extract was determined through the disco-diffusion method. The effectiveness of *O. vulgare* essential oil was tested *in vitro* for *Streptococcus mutans* biofilm using colorimetric analysis.

Results: *O. vulgare* essential oil inhibited the growth of *S. mutans* biofilm by 98% compared with unexposed control bacteria ($p < 0.05$). Five samples of anti-caries gel (ACDG1, ACDG2, ACDG3, ACDG4, ACDG5) were formulated using the obtained oregano essential oil and extract. Based on the microbiological study results, the ACDG1 and ACDG3 gel samples exhibited high antimicrobial activity against the gram-positive bacterial strains of *Staphylococcus aureus* and *Bacillus subtilis* and the yeast fungus *Candida albicans* and moderate antimicrobial activity against the gram-negative strains of *Escherichia coli* and *Pseudomonas aeruginosa*.

* Corresponding address: Gogol str., 40, Karaganda, 100000, Kazakhstan.
E-mail: Karlito42@mail.ru (K.Zh. Badekova)

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Conclusion: Based on the results of this study, the ACDG3 sample may be considered an anti-caries gel owing to its high antimicrobial activity. This sample has good organoleptic properties compared to other samples, produces relatively high antimicrobial activity, and guards against cariogenic biofilms of *S. mutans*.

Keywords: Anti-bacterial agent; Biofilm; Gel; *Origanum*; Plant extract; *Staphylococcus aureus*

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Introduction

Despite great advances in the prevention and treatment of dental caries, this disease is a serious public health problem in most countries, which is complicated by the higher cost of restorative treatment and new evidence of the relationship between caries and a number of systemic diseases.[1]

Tooth decay (dental caries) is a disease that is most often induced by a high rate of the cariogenic bacteria *Streptococcus mutans*, which form a biofilm resulting in dental plaque.[2] The increasing antibiotic resistance of pathogenic bacteria has led researchers to a focus on ethnopharmacology as an alternative therapeutic approach.[3] These data confirm that essential plants have powerful antiseptic and antiviral properties which inhibit the formation of mature biofilms.[4]

In the search for a new effective anti-caries agent from plant essential oils, this study determined the effectiveness of the eugenol and thymol monoterpenoids against polymicrobial biofilms (*Streptococcus sanguinis, Lactobacillus acidophilus, Actinomyces viscosus*, *S. mutans*).[5] Research findings have shown that eugenol is effective against biofilms of two oral pathogens (*Candida albicans* and *S. mutans*) *in vitro*, and can also be used in synergy with azithromycin and fluconazole in the control of oral infections.[6]

Citrus aurantium L. essential oil has also reduced the growth of *S. mutans* and can be used to prevent/control oral infections.[7] Essential oils extracted from oregano, carnation, thyme, and cinnamon plants feature the greatest antibacterial activity against *S. mutans*. Toothpastes containing essential oils of these plants have also been found to be capable of completely destroying *S. mutans* biofilms.[8]

Plant extracts are often used in dentistry because of their high anti-inflammatory and antimicrobial properties and as antioxidants, antiseptics, analgesics, fungicides, bactericides, and antivirals.[9] Extracts and essential oils of some plant species can control skin-related microorganisms, dental caries, and bacteria. Recent studies have revealed natural remedies to be effective in preventing and treating oral diseases, namely plaque and caries.[10] Globally, the use of medicinal herbs for therapy is growing in intensity every day.[11]

In modern pharmaceutical and dental practice, gels and ointments with antibacterial and anti-inflammatory effects are often used. They have prolonged effects, employ simple techniques, and are easy to use.[12]

The Kazakhstan drug register includes only 38 dental preparations, eight of which are gels.[13] Moreover, only one of these eight gels, Kamesan, is produced in Kazakhstan. Therefore, the development of a new effective antimicrobial and anti-caries agent based on homegrown plant products is very important. This study aimed to solve one of the most urgent priorities of the Republic of Kazakhstan – import substitution, or the creation of new domestic products for the treatment and prevention of oral bacterial infections and caries.

The grounds for the development of this drug product based on essential oil and ethanol extract of *O. vulgare* were a wide range of biologically active substances with multi-functional pharmacological effects, particularly, pronounced bactericidal and anti-caries ones. Plant essential oils and extracts show a relatively high antimicrobial effect against known bacterial and fungal pathogens, which suggests their use for oral hygiene. For researchers, this suggests the study of the component formulation of oregano essential oil growing in Kazakhstan, its antimicrobial and anti-caries effect as well as the formulation of a new dental gel based on it.

This study aimed to test the effectiveness of *O. vulgare* essential oil against *S. mutans* biofilm, to produce an anti-caries gel with oregano extract and essential oil and then to determine its antimicrobial activity *in vitro*.

Materials and Methods

Study design

This preclinical study lasted 105 days. During this period, we tested the effectiveness of *O. vulgare* essential oil against *S. mutans* biofilm and formulated an anti-caries gel with oregano extract and essential oil. The antimicrobial activity of the gel on microbial strains was determined *in vitro*.

Plant material

Plant products, namely, the aerial portion of *O. vulgare*, were collected in the vicinity of Shchuchinsk, Akmola region, in the flowering period.

Extraction of O. vulgare essential oil

An antimicrobial dental gel involves for its formulation an oregano essential oil which is extracted by hydro-distillation from an air-dry mass of plant product using a Clevenger device (‘Khimlaborpribor,’ Russia). To collect the essential oil, hydro-distillation was performed over 3 h using hexane as a solvent.[14] The formula and components of the oregano essential oil were determined by gas chromatography/mass spectrometry[15] (Agilent GC System 7890A, Agilent Technologies Inc., USA). The EM yield was 0.5%. The carvacrol in the oil reached 86%.

The ethanolic extraction of oregano was performed. Fifty grams of dry cut oregano were placed in 1 L of 96% ethyl alcohol (1:20) and infused for 24 hours. Once filtered, the extract was concentrated in the rotary evaporator (Ulab UL-1100, China), and the ethanol–water mixture was distilled. The concentrated extract was evaporated in a porcelain evaporating pan placed in a water bath at 80 °C until a thick extract resulted. It produced a thick dark green extract with a pleasant odour and a yield of 6.21%. The main component, according to high-performance liquid chromatography,[16] (Agilent 1260 Infinity, Agilent Technologies Inc., USA) was rosmarinic acid.

Biofilm formation, treatment, and analysis by colorimetric assay

S. mutans strain UA159 (ATCC 700610) was used to produce the biofilm on a polystyrene surface of the flat-bottom wells of 24-well cell culture plates (Sarstedt, Germany). The stock concentration of *O. vulgare* essential oil (100 mg/ml) was made in the pure dimethyl sulfoxide (DMSO) (Sigma–Aldrich, USA). For *S. mutans* biofilm development and treatment, Bacto™ Todd Hewitt broth (BD Bio Sciences, USA) supplemented with 1% sucrose was dispensed into the plate wells followed by the addition of the solution of *O. vulgare* essential oil at final concentrations of 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, and 10 mg/ml. Then, *S. mutans* (1.6×10^8 cells/ml) were inoculated into the plate wells at a final dilution of 1:100, and the plates were incubated under anaerobic conditions (95% N_2 and 5% CO_2) at 37 °C for 24 h. In this study, the plate wells without *S. mutans* served as blank controls, while *S. mutans* cells without any treatment and treated only with DMSO (2%, 4%, 6%, 8%, 10% (v/v)) were used as experimental controls.

After 24 h of incubation, the biomass of *S. mutans* biofilm was quantified using a colorimetric method. The broth was briefly removed from the plates, the wells were washed with distilled water, and the adherent biofilm at the bottom of the wells was fixed by applying 95% ethanol. The dried biofilm was stained using 0.01% crystal violet solution (Merck, USA), followed by extraction of the bound dye with 33% acetic acid solution (Merck, USA). Microplate reader-spectrophotometer (Dynex Technologies, USA) set to the wavelength of 595 nm was used to measure optical density of the extracted dye solution from the samples.

Gel preparation

For 100.0 g of gel, the measured sodium carboxymethyl cellulose (Na CMC) volume was placed in a 500-ml beaker and mixed with cold purified water, stirred, and infused. It was then left to thicken over 15–20 min. The solution was then stirred with the stirrer at 50–100 rpm until completely dissolved. The oregano extract was then dissolved in a separate container with a measured amount of glycerine, and the measured amount of oregano essential oil and other substances were added drop-wise. The prepared solution was added to the gel base with the stirrer turned on at 100 rpm and stirred until a homogeneous mass was produced.

Several samples (five models) of the anti-caries gel were prepared *in vitro*, using sodium CMC as a gelling agent. According to numerous studies, the best option for a gel base is an aqueous solution of sodium CMC with the addition of glycerin as a plasticizer, which has good structural and mechanical properties, releasing ability and good compatibility with both chemical and plant medicine.[17] As an active plant-derived ingredient, it contains ethanol extract and oregano essential oil.

In our formulation, ethanol extract and oregano essential oil were used as active ingredients and preservatives. The main components of essential oils are terpenoid compounds: terpenes C10H16 and their oxygen-containing derivatives.[18] According to the data, carvacrol, as the main component of oregano essential oil, is a preservative.[19] The *O. vulgare* extract has also been noted to have similar properties.[20] In other words, oregano extract and essential oil can be used as natural food preservatives. The formulation of the five samples of dental anti-caries gel (ACDG1, ACDG2, ACDG3, ACDG4, ACDG5) is shown in Table 1.

Assay for the determination of antimicrobial activity in vitro

The formulated gel samples were screened for antimicrobial activity by the disk diffusion method.[21] The antimicrobial activity of the above samples was studied for strains of the gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), for the gram-negative strains *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), and for the yeast fungus *Candida albicans* (ATCC 10231). Comparators are benzylpenicillin for bacteria and nystatin for the yeast fungus *C. albicans*.

For the study, it was necessary to prepare a suspension with a standard number of viable bacterial cells. This was inoculated with a lawn on the medium surface in Petri dishes, and 0.01 ml anti-caries gel was applied to sterile discs made of filter paper. Discs with preparations were applied to the inoculation at 2.5 cm from the centre of the dish circle–wise (6 disks per dish). The inoculations were incubated over 24 h at 36 °C. Once incubated, a uniform bacterial lawn around the disks facilitated the formation of zones of complete and partial inhibition of bacterial growth. The findings were counted by measuring the diameter of the growth inhibition zones.

Statistical analysis

Data obtained from the colorimetric assay were evaluated with SPSS software version 23.0 (IBM Corporation, USA). The differences between the control (untreated) and treatment groups were assessed by applying a one-way analysis of variance (ANOVA), followed by a post hoc least-significant difference test for multiple comparisons. The figures indicate the mean and standard errors. The values were considered statistically significant when the p value was less than 0.05.

Results

Inhibitory effect of O. vulgare essential oil on S. mutans biofilm formation

The assessment of the effectiveness of the *O. vulgare* essential oil for inhibition of *S. mutans* biofilm formation using colorimetric assay revealed its capacity to considerably

suppress biofilm development on the polystyrene surface in the 24-well cell culture plates (Figure 1). Treatment with the *O. vulgare* essential oil at a concentration of 2 mg/ml resulted in only a slight reduction in biofilm production at the bottom of the wells in the 24-well cell culture plates. However, concentrations of 4 mg/ml, 6 mg/ml, 8 mg/ml, and 10 mg/ml of the *O. vulgare* essential oil almost completely inhibited *S. mutans* biofilm accumulation at the bottom of the wells in the 24-well cell culture plates.

As shown in Figure 2, the *O. vulgare* essential oil at a concentration of 2 mg/ml caused an insignificant reduction of 9% in *S. mutans* biofilm biomass versus the untreated control bacteria ($p > 0.05$, as determined quantitatively using a one-way ANOVA, followed by a post hoc least-significant difference test). However, the *O. vulgare* essential oil at concentrations of 4 mg/ml, 6 mg/ml, 8 mg/ml, and 10 mg/ml significantly decreased *S. mutans* biofilm biomass by 98% compared to the untreated control bacteria ($p < 0.05$, as determined quantitatively using a one-way ANOVA, followed by a post hoc least-significant difference test).

The inhibitory activity of *O. vulgare* essential oil did not occur in a dose-dependent manner. Of note, the DMSO concentration of 2% did not significantly decrease the biofilm biomass in comparison with the untreated bacteria ($p > 0.05$, as determined quantitatively using a one-way ANOVA, followed by a post hoc least-significant difference test). On the other hand, treatment of bacterial cells with DMSO alone caused a significant reduction in *S. mutans*

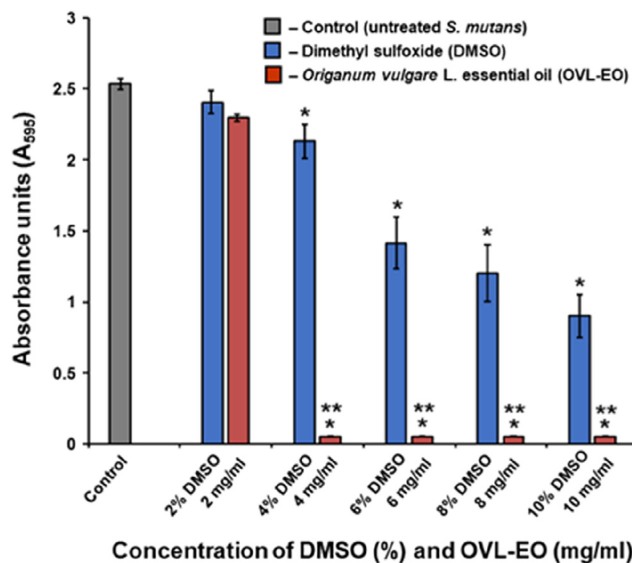

Figure 2: Quantities of *Streptococcus mutans* biofilm biomass following 24 h of incubation within Todd Hewitt broth in the presence of 1% sucrose and various concentrations of *Origanum vulgare* essential oil and dimethyl sulfoxide (DMSO). Values are shown as the mean ± standard error obtained from a single experiment (n = 2–26). *$p < 0.05$ versus the control; **$p < 0.05$ versus the DMSO.

Table 1: Composition of anti-caries gel samples (100.0 g).

Ingredient name	Purpose	Samples				
		ACDG1	ACDG2	ACDG3	ACDG4	ACDG5
Origanum vulgare extract (g)	Active substance	6.0	6.0	6.0	–	6.0
Origanum vulgare essential oil (ml)	Active substance	12.0	12.0	12.0	12.0	–
Sodium CMC (g)	Gel base	22.0	22.0	22.0	22.0	22.0
Glycerine (ml)	Plasticizing agent	20.0	20.0	20.0	20.0	20.0
Twin 80 (ml)	Stabiliser	–	12.0	–	–	–
NaOH 10%	Acidity regulator	2				
Sweetener (g)	Corrigent		–	3	1	3
Distilled water (ml)	Solvent	100	100	100	100	100

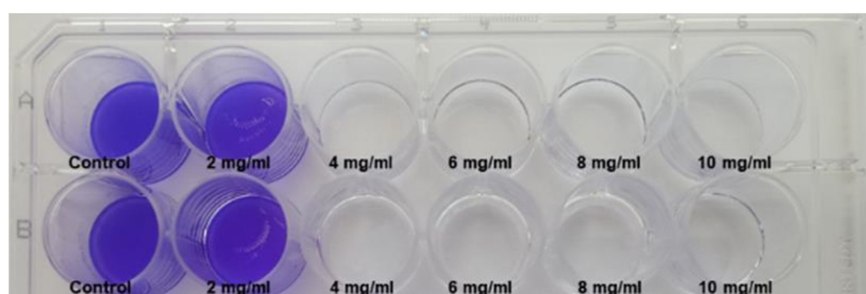

Figure 1: Suppressive activity of *Origanum vulgare* essential oil against *Streptococcus mutans* biofilm formation on a polystyrene surface. The figure represents the segment of the 24-well cell culture plate showing *S. mutans* biofilm stained with 0.01% crystal violet solution following 24 h of incubation within Todd Hewitt broth in the presence of 1% sucrose and various concentrations of *O. vulgare* essential oil. In the A and B rows: untreated *S. mutans* biofilm (control); *S. mutans* biofilm treated with 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, and 10 mg/ml of *O. vulgare* essential oil.

Table 2: Antimicrobial activity of the 5 investigated samples of anti-caries gel (inhibition zone measured in mm).

Microbes	ACDG1	ACDG2	ACDG3	ACDG4	ACDG5	Benzyl penicillin	Nystatin
Staphylococcus aureus	27 ± 1	10 ± 1	25 ± 1	_	9 ± 1	20 ± 1	
Bacillus subtilis	30 ± 1	_	25 ± 1	_	9 ± 1	25 ± 1	
Escherichia coli	20 ± 1	_	16 ± 1	_	_	20 ± 1	
Pseudomonas aeruginosa	16 ± 1	–	16 ± 1	_	_	12 ± 1	_
Candida albicans	35 ± 1	15 ± 1	25 ± 1	10 ± 1	10 ± 1		22 ± 1

biofilm biomass, versus the untreated bacteria, at DMSO concentrations of 4%, 6%, 8%, and 10% ($p < 0.05$, as determined quantitatively using a one-way ANOVA, followed by a post hoc least-significant difference test). This DMSO activity was induced in a dose-dependent manner. Importantly, the suppressive effect of *O. vulgare* essential oil at concentrations of 4 mg/ml, 6 mg/ml, 8 mg/ml, and 10 mg/ml was significant compared to the corresponding concentrations of DMSO ($p < 0.05$, as determined quantitatively using a one-way ANOVA, followed by a post hoc least-significant difference test). This indicates that *O. vulgare* essential oil exhibited inhibitory activity against *S. mutans* biofilm formation independently from DMSO, causing a considerably greater effect than DMSO alone.

Antimicrobial activity of the anti-caries gel

The antimicrobial activity of the samples was assessed based on the diameter of the growth inhibition zones of the test strains (mm). The diameter of the growth inhibition zones of less than 10 mm and confluent growth in the dish were assessed as the deficiency of antimicrobial activity, 10–15 mm=low activity, 16–20 mm–moderate activity, and more than 20 mm–high activity.

Table 2 shows that the samples of the ACDG1 and ACDG3 gel manifest pronounced activity against *S. aureus, B. subtilis*, and *C. albicans* and moderate activity against *E. coli, P. aeruginosa*. A weak antimicrobial activity of the ACDG2 sample against *S. aureus* and *C. albicans* was revealed. The results are two and one and a half times less compared to the control. The ACDG4 and ACDG5 samples show a weak antimicrobial activity against *C. albicans*, the results are two times less than in the control.

Optimal formulation of the dental gel

The study of the properties of the various dental gel formulations made from Samples 1–5 showed the ACDG3 sample to be optimal in terms of consistency, ease of use, and treatment efficacy. The ACDG4 and ACDG5 samples were noted to have insufficient adhesive, coating abilities, and organoleptic properties. The ACDG1 and ACDG2 samples were too thin, which made it difficult to apply the gel evenly. Therefore, the ACDG3 sample was accepted as the optimal formulation of the dental gel. The composition of the ACDG3 sample is shown in Table 3.

Table 3: ACDG3 sample composition (100.0 g).

S.no.	Ingredients	Mass
1.	*Origanum vulgare* extract (g)	6.0
2.	*Origanum vulgare* essential oil (ml)	12.0
3.	Sodium CMC (g)	22.0
4.	Glycerine (ml)	20.0
5.	Sweetener (g)	3.0
6.	Distilled water (ml)	100

Discussion

The findings confirmed the experimental data that oregano essential oil has an anti-caries effect; that is, it can significantly inhibit *S. mutans* biofilm, which is the main cariogenic microorganism. A previous study revealed thymol and carvacrol monoterpenes extracted from the *O. vulgare* essential oil to exhibit relatively high bactericidal and antibiotic filter activity against *S. mutans* that can serve as a green alternative to control dental caries.[22]

Compared with *O. vulgare* essential oil collected in Iran, whose main components are limonene and myrcene, the main component of the essential oil of oregano growing in Kazakhstan is carvacrol. Oregano essential oil and its main components have been shown to have high antibiotic film and antibacterial properties and can be used to produce new plant-based preparations.[23]

Depending on the place of growth, different chemical formulas have been discovered in the above-mentioned essential oils of the same species of *O. vulgare,* but screening for the destruction of *S. mutans* biofilms has revealed that these monoterpenoids of essential oils (thymol, carvacrol, limonene, myrcene, etc.) can be qualified as broad-spectrum antimicrobial substances that affect microorganisms of oral diseases.

O. vulgare was found to manifest antimicrobial activity against a wide range of bacteria, including antibiotic-resistant species. Moreover, it is able to affect gram-positive and gram-negative bacteria, and yeast fungus.[24] The findings of this study delivered on our expectations and confirmed the validity of previous studies conducted in other countries. The study findings are essential, as they prove that the oregano that grows in the Republic of Kazakhstan has a high anti-caries and antimicrobial effect, which can form the base of new effective medicines for the treatment of dental diseases.

The data obtained from the tested gel samples facilitate a clearer understanding of the effects of changes when only one of the active substances is added, or the formulation is changed by adding a stabiliser, which weakens antimicrobial activity. This suggests the inhibitory properties of these compounds and the need to test the sample, even if only one ingredient is changed.

The above experimental findings allow for the acceptance of the resulting optimal composition of oregano-based ACDG3 gel as an effective import-substituting agent for the treatment and prevention of common oral bacterial

infections and caries and the expansion of the range of effective and safe domestic dental drugs.

The resulting data are very important, as they prove the effectiveness of the formulated gel against caries and indicate the high antimicrobial properties of this dental gel, which, in turn, confirms the feasibility of using oregano as the main active component. Moreover, such studies are essential to the further formulation of new medicines based on domestic herbal plants.

The rheological and toxicological properties of this anti-caries dental gel will be further investigated in future studies.

Study limitations

It is necessary to emphasise that the colorimetric assay used for quantification of the biofilm biomass has some limitations, which include sample destruction and a lack of dimensional information about the biofilm. Therefore, the inhibitory effect of *O. vulgare* essential oil on *S. mutans* biofilm formation should be demonstrated and confirmed using other methods. In this regard, scanning electron microscopy and/or optical profilometry techniques should be implemented in our further studies, enabling the three-dimensional visualisation of the biofilm on different surfaces (e.g., glass, tooth surfaces) and the evaluation of the biofilm thickness and its surface roughness parameters. In addition, the application of real-time reverse transcription-PCR would allow to assess quantitatively expression of biofilm-associated genes (e.g. genes encoding glucosyltransferases B, C, and D) in *S. mutans* bacteria, providing more insights into the biofilm-inhibiting mechanism of *O. vulgare* essential oil.

Conclusions

This study showed that ACDG3 among the resulting gel samples was optimal for an anti-caries dental gel with oregano extract and essential oil. It may also be concluded that the above-mentioned gel sample has high antimicrobial and antifungal activity; that is, the gel may completely clean the surface of the teeth and prevent caries and the growth of microorganisms, thereby facilitating good oral hygiene. Moreover, the main active component of the dental gel, the essential oil *O. vulgare*, could inhibit the formation of biofilms caused by *S. mutans*.

Recommendation

The authors recommend undertaking resource studies of *O. vulgare* in Kazakhstan, which will determine the distribution and stocks of herbal substances, conducting resource mapping, and drawing up scientifically based recommendations for the regional planning of procurements.

Source of funding

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

The research did not involve any testing in animals or humans.

Authors' contributions

BKZh conceived and designed the study, conducted research, provided research materials, and collected, organised and analysed the data. SMK and ASB edited the manuscript. AGA and KT wrote the final draft and provided logistical support. All authors critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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References

1. Lapina N, Lobach O. Modern aspects of the etiology and pathogenesis of dental caries. **Clin Dent 2017**; 84(4): 76–79. Russian.
2. Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, et al. The biology of *Streptococcus mutans*. **Microbiol Spectr 2019**; 7(1): 10.
3. Karadağlıoğlu Öİ, Ulusoy N, Başer KHC, Hanoğlu A, Sık İ. Antibacterial activities of herbal toothpastes combined with essential oils against Streptococcus mutans. **Pathogens 2019**; 8(1).
4. Ben Abdallah Fethi, Lagha Rihab, Gaber Ahmed. Biofilm Inhibition and Eradication Properties of Medicinal Plant Essential Oils against Methicillin-Resistant *Staphylococcus aureus* Clinical Isolates. **Pharmaceuticals (Basel). 2020**; 13.
5. Utami DT, Pratiwi SU, Haniastuti T, Hertiani T. Eugenol and thymol as potential inhibitors for polymicrobial oral biofilms: an *in vitro* study. **J Int Oral Health 2021**; 13: 45–52.
6. Jafri H, Banerjee G, Khan MSA, Ahmad I, Abulreesh HH, Althubiani AS. Synergistic interaction of eugenol and antimicrobial drugs in eradication of single and mixed biofilms of Candida albicans and Streptococcus mutans. **AMB Express 2020 Oct 19**; 10(1): 185.
7. Benzaid C, Belmadani A, Tichati L, Djeribi R, Rouabhia M. Effect of *Citrus aurantium* L. Essential oil on *Streptococcus mutans* growth, biofilm formation and virulent genes expression. **Antibiotics (Basel) 2021**; 10(1): 54.
8. de Oliveira Carvalho I, Purgato GA, Píccolo MS, Pizziolo VR, Coelho RR, Diaz-Muñoz G, et al. In vitro anticariogenic and antibiofilm activities of toothpastes formulated with essential oils. **Arch Oral Biol 2020**; 117: 104834.
9. Ravi SB, Nirupad S, Chippagiri P, Pandurangappa R. Antibacterial effects of natural herbal extracts on *Streptococcus*

mutans: can they Be potential additives in dentifrices? **Int J Dent 2017**; 5.

10. Carrol DH, Chassagne F, Dettweiler M, Quave CL. Antibacterial activity of plant species used for oral health against *Porphyromonas gingivalis*. **PloS One 2020**; 15(10):e0239316.
11. Msomi NZ, Simelane MBC. Herbal medicine. **IntechOpen 2018**; 10: 5772.
12. Tokmakova SI, Lunitsyna YuV, Kiyashchenko YuV, Kozlova KS, Barg EV. Comparative assessment of the antimicrobial activity of dental gels. **Dent Prob 2014**; 1.
13. National center for expertise of medicines and medical devices of the Committee for medical and pharmaceutical control of the Ministry of health of the Republic of Kazakhstan, accessed 7 april 2021, https://www.ndda.kz/category/gos_reestr_excel.
14. Dao TP, Tran TH, Nhan NPT, Quyen NTC, Tien LX, Anh TT, et al. Optimization of essential oil yield from Vietnamese green pepper (Piper nigrum) using hydro-distillation method. **IOP Conf Ser Mater Sci Eng 2020**: 736. 022039.
15. Simirgiotis MJ, Burton D, Parra F, López J, Muñoz P, Escobar H, Parra C. Antioxidant and antibacterial capacities of *Origanum vulgare* essential oil from the arid andean region of Chile and its chemical characterization by GC-MS. **Metabolites 2020**; 16: 414. 10(10).
16. Zoccali M, Giocastro B, Bonaccorsi IL, Trozzi A, Tranchida PQ, Mondello L. In-depth qualitative analysis of lime essential oils using the off-line combination of normal phase high performance liquid chromatography and comprehensive two-dimensional gas chromatography-quadrupole mass spectrometry. **Foods 2019**; 8(11): 580.
17. Ryumina TYe, Golovanenko AL, Tretyakova YeV, Berezina YeS, Alekseyeva IV, Pershina RG. Biopharmaceutical research of gels and medicinal films for the treatment of enamel caries. **Modern Prob sci Educ 2015**; 4: 549. Russian.
18. Fongang FYS, Bankeu KJJ. Terpenoids as important bioactive constituents of essential oils. **IntechOpen 2020**; 10: 5772.
19. Marinelli L, Di Stefano A, Cacciatore I. Carvacrol and its derivatives as antibacterial agents. **Phytochemistry Rev 2018**; 10: 1007.
20. Veenstra JP, Johnson JJ. Oregano (Origanum vulgare) extract for food preservation and improvement in gastrointestinal health. **Int J Nutr 2019**; 3(4): 43−52.
21. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: a review. **J Pharm Anal 2016**; 6(2): 71−79.
22. Khan S, Khan M, Ahmad J, Wahab R, Abd-Elkader O, Musarrat J, et al. Thymol and carvacrol induce autolysis, stress, growth inhibition and reduce the biofilm formation by *Streptococcusmutans*. **AMB Express 2017**; (1).
23. Hejazinia F, Fozouni L, Azami NS, Mousavi S. The antibiofilm activity of oregano essential oil against dental plaque-forming *Streptococcus mutans* in vitro and in vivo. **J Kerman Univ Med Sci 2020**; 24(3):e107680.
24. Leyva-López N, Gutiérrez-Grijalva EP, Vazquez-Olivo G, Heredia JB. Essential oils of oregano: biological activity beyond their antimicrobial properties. **Molecules 2017**; 22(6): 989.

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