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Comparison between mastic gum resin extract and chlorhexidine mouthwash in the prevention of biofilm formation on titanium dental implants

Ranj Nadhim Salaie*

Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Tishk International University, Erbil, Iraq

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ABSTRACT

The biofilm formation around dental implant abutment is considered the main cause of peri-implant infection. The use of antimicrobial mouthwash might potentially reduce biofilm formation and subsequent infection. This study aimed to evaluate the antibiofilm properties of mastic gum resin extract (MGRE) against *S. Mutans* biofilm on the surface of titanium dental implant discs. This study used grade five (medical grade) titanium dental implant discs measuring 15 mm in diameter. The substances in the MGRE were analyzed by gas chromatography mass spectrometry (GC-MS) which found 12 detectable chemicals in MGRE. In this study, *S. mutans* was collected around the healing abutment of a recently placed dental implant and then cultured on titanium discs (*in vitro*). After bacterial growth on the titanium specimens for 24 hours, they were subjected to either chlorhexidine gluconate (CHX), (n = 6) or MGRE (n = 6). The antibacterial activity tests showed that both CHX and MGRE significantly inhibited bacterial growth compared to the negative control, the lactate production and turbidity measurements were significantly lower in MGRE and CHX compared to the control ($p \leq 0.05$). Regarding the antibiofilm activity, both treatments showed significantly less turbidity in their biofilm compared to the control. Moreover, scanning electron microscopy (SEM) images showed that there were very limited adherence cells on the titanium implant discs in treatment groups, while there were confluent and attached bacterial cells in the control. The MGRE showed an antimicrobial property against *S. mutans* indicating that it has a potential for clinical use as a mouthwash.

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Introduction

Peri-implant mucositis is one of the risk factors that threaten the success of dental implant treatment. Peri-implant mucositis, if untreated, can lead to peri-implantatis which consequently results in peri-implant bone loss and implant failure. Peri-implant mucositis is defined as an inflammatory lesion of the mucosa surrounding an endosseous implant without loss of supporting peri-implant bone (1). Many factors can be considered as risk indicators for peri-implant mucositis, one of them is the presence of plaque biofilm (2, 3). Peri-implant mucositis develops from healthy peri-implant mucosa resulting from the accumulation of bacterial biofilms around osseointegrated dental implants (3). The prognosis of peri-implant mucositis might take longer than gingivitis. It was found that the clinical reversibility of peri-implant mucositis can take longer than 21 days (4). However, with consistent oral hygiene practice, the biofilm-induced per—implant mucositis can be significantly improved. For example, it was found that the gingival index of peri-implant mucositis significantly reduced after oral hygiene measures compared to the periodontal side (4).

Researchers have tried to prevent or treat peri-implant mucositis in various ways. Interventions have been used to remove the peri-implant biofilm from contaminated implant surfaces (5). Studies have used air powder abrasion using glycine powder or chitosan brush along with mechanical debridement. However, no significant clinical benefits regarding BI and BoP were observed compared to the control (6, 7). Moreover, a randomized controlled clinical trial was conducted to investigate the effect of photodynamic therapy addition to mechanical debridement on BI and BoP, it was found that the intervention did not demonstrate a significant difference in BI and BoP compared to control (8, 9). Another randomized controlled clinical trial was conducted to study the effect of diode laser as an adjunctive to mechanical debridement on BI and BoP, the results showed that laser treatment did not induce a significant improvement in BI and BoP compared to the control (10,11). Furthermore, some studies have used adjunctive local antiseptics, for example, studies have used CHX gel and mouth rinse as an adjunctive measure to treat peri-implant mucositis, and results showed no significant improvement compared to control (12,13). Another research used sodium hypochlorite as an adjunctive therapy but there was no significant improvement compared to the control (14).

Based on the mentioned studies, the use of mouthwashes as an adjunctive therapy has not shown a significant improvement in peri-implant clinical parameters. However, their in-vitro antibacterial efficacy is well documented, but using other interventions (antimicrobial agents) might provide a better antimicrobial activity. Research showed that the antibacterial activity of non-thermal atmospheric pressure plasma against biofilms of *S. mutans* grown on

* Corresponding author. Email: ranj.nadhim@tiu.edu.iq

titanium dental implant discs was significantly more than CHX (15). This can be explained by the fact that oral microbes might have developed resistance against CHX which is most commonly as an antimicrobial mouthwash.

Recently, natural, plant-driven products have shown potential antibacterial and anti-inflammatory activity (16-18). Herbal mouthwashes have shown similar ant-plaque activities as compared to the standard mouthwash CHX (19). MGR is an exudate from *Pistacia atlantica* tree, its antioxidant and anticancer activities are well known, and an in-vitro study has shown the efficacy of MGR in arresting the cell cycle of colon cancer cell line at the G2/M phase indicating that the resin can be used to prevent colon cancer (20). Moreover, studies have shown that MGR possesses an excellent antibacterial properties against *Helicobacter pylori* (21). Regarding the oral microbiota, a study was conducted to investigate the antibacterial activity of mastic chewing gum (traditional chewing gum made from MGR) against *S. mutans*, the results showed significantly lower *S. mutans* in saliva samples collected after chewing mastic gum compared to control (22). Taken together, to reduce the bacterial resistance to the currently used mouthwashes, eliminate their undesirable side effects (e.g.; teeth staining) and preserve oral normal flora, MGRE (in a liquid form) can be used as a mouthwash to decrease peri-implant microbiota. Thus, this study aimed to investigate the antibacterial activity of MGRE against isolated *S. mutans* (early colonizer) around dental implants inside the oral cavity.

Materials and Methods

Specimen preparation

This study used medical, grade five titanium alloy (Ti6Al4V) discs which are most commonly used for dental implants. The circular-shaped discs are 15 mm in diameter and 1 mm in thickness created by laser cutting and polished with sandpapers of (800–1200 grit) using a rotary tool (Grinder–Polisher, Buehler, UK Ltd, Coventry, England). For the final polish, six and one microns of diamond solution (Diamond solution, Kemet International Ltd, UK) were applied. Finally, the specimens were cleaned using an alkaline solution and 5% HCl as described in (23, 24).

Preparation of MGRE

MGR was collected from *Pistacia atlantica* tree located in Qandeel mountain Kurdistan region of Iraq (Figure 1). The resin was collected from the tree and then boiled for 30 minutes. The aqueous extract from the resin was collected. This aqueous extract has long been used traditionally to treat gastric upset.

Figure 1. Matic gum resin collected from Pistacia atlantica tree. Note the clay pots attached to the tree to collect the resin.

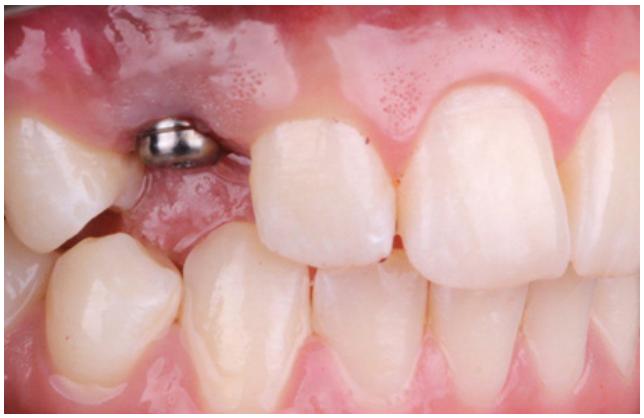

Figure 2. Gingival former (healing abutment) around which the microbial swab was collected.

Chemical content analysis of MGRE using GC-MS

The chemical content of the extract was identified using GC-MS. The analysis was carried out with an Agilent 7000 Triple Quad, GC 7890A. GC oven temperature was programmed to 220 ◦C at a rate of 4 ◦C/min and kept constant at 220 ◦C for 10 min and then programmed to 230 ◦C at a rate of 1 ◦C/min. The ion source temperature was 230 °C and the scan mode of the mass spectrum of 25-800 atomic mass units. The mass spectra were recorded at 70 eV.

Isolation and identification of *S. mutans* around dental implant abutment

The bacterial swab was collected around a dental implant healing abutment of 28-year-old female patient (Figure 2). The sample was transferred to the laboratory and cultured on blood agar then incubated for 24 hours at 37 °C. After that, a coolly was identified on the blood agar which was later identified using the VITEK II device (bio-Merieux, North Carolina, USA).

Assessment of the antibacterial activity using the disc diffusion method

The antibacterial activity of MGRE was tested using the disc diffusion method which is a conventional method used for testing microbial growth sensitivity against antimicrobial agents. In the current study, 6 mm diameter filter papers were immersed in MGRE (n=6) and CHX, (n=6) for 3 hours, after that, the conditioned filter paper was transferred to Mueller Hinton agar where *S. mutans* were newly sub-cultured, and agar plates were the unit of replication. After 24-hour incubation, the agar plates were examined for the presence of microbial growth inhibition zone around the filter papers.

Preparation of bacterial suspension

The microbial samples which were isolated from the dental implant abutment were put in blood agar and then incubated for 24 hours at 37 °C to acquire bacterial growth. Then a swab of the microbial colony in the blood agar was cultured in brain heart infusions (BHIB). The sample in BHIB was put in anaerobic jars and incubated at 37 °C for 24 hours for activation.

Experimental design

The main experiment in this study tested the antibacterial and antimicrobial activity of MGR and CHX against *S. mutans* grown on titanium dental implant discs. The experimental groups were negative control, Treatment (MGRE) and positive control (0.2% CHX). Basically, *S. mutans* in 1.5 BHIB was added to the titanium discs in sterile glass tubes and incubated overnight at 37 °C in an anaerobic jar. After 24 hours, the BHIB was removed and 5 ml of MGRE (n=6) and 0.2% CHX (n=6) were added to the specimens for 60 seconds. The test solutions were then washed away, and the specimens were rinsed three times with ultrapure water. After that fresh BHIB was added to the specimens and incubated for 24 hours. Finally, the turbidity of BHIB and the biofilm were measured. Another triplicate of control and treatment and positive control was also prepared for assessing bacterial morphology after 24 hours under the SEM.

Optical density measurement for bacterial growth assessment

The turbidity test is commonly used to assess bacterial growth in the media, the increased turbidity corresponds the bacterial growth. The procedure started by taking 100 µl aliquots of the nutrient broth of blank, negative control, treatment and positive control groups were added to a 96-well plate with a flat bottom and a lid and then 100 µl of the fresh BHIB was added to each well. The 96-well plate was then placed in a plate reader (BioTek ELX800) and the absorbance values were read at 630 nm to determine the turbidity.

Assessment of biofilm growth on titanium implant discs

The antibiofilm assay used in this study followed the protocol used (25). After 24 hours, the media was removed, and the biofilm was stained for 10 minutes using 1% crystal violet. The stain intensity was used to determine the biofilm strength. After staining, the specimens were rinsed with distilled water to remove excess stain and left at room temperature to dry. 3 mL of ethanol was then added on each specimen to detach the biofilm. Finally, the ethanol solution including the biofilm was withdrawn and placed in 96 well-plated and the optical density was measured using a plate reader (BioTek ELX800) at 630 nm.

Investigating the morphology and confluence of *S.mutans* on titanium discs using SEM

SEM was used to visualize the morphology of the bacterial cells after the experiment. The bacterial cells were examined *in situ* on the titanium discs. After the experiment, the media were discarded, and the specimens were washed with phosphate buffer. After that, the bacterial biofilm on the discs was immersed in ethanol solutions (30, 50, 70, and 95%) for 20 min each then 100% ethanol for 1 h. Later, the specimens were left to dry overnight and then sputter-coated with chromium. The bacterial cells on the discs were then examined under SEM for morphology and confluence.

Statistical analysis

Data are expressed as mean ± S.E.M. and analyzed using stat graphics version 16. To locate the significant difference between the groups, data were subjected to one-way ANOVA, then Tukey's test. All statistical analyses used a 95% confidence limit, p values < 0.05 were considered statistically significant.

Results

Chemical analysis of MGRE

The chemical content of the aqueous MGRE was analyzed using GC-MS. The analysis found 12 chemical structures through 12.3-18.3 min analysis (Figure 3). The names of chemical structures with their start, end and real time are shown in (Table 1).

Microbiological analysis of the peri-implant sample

The peri-implant abutment swab was cultured in blood agar for 24 hours, then, the formed bacterial colony was analyzed by VITEK II for microbial identification. The result showed that the colony was *S. mutans* having 97% percentage probability.

Table 1. Name (structure) of the chemicals detected in MGRE using GC-MS.

Peak Name	Start	Real Time	End
α-Pinene	12.43	12.5	12.673
Diepoxyhexadecan	14.8	14.9	15.06
3-Cyclohexene-1-methanol, 2-hydroxy-alpha, alpha,4-trimethyl-	15.21	15.26	15.32
5-Bornanedione	15.49	15.53	15.62
α-Linolenic acid	15.78	15.83	15.92
Boronia butenal	16.31	16.35	16.43
Acetate	16.89	16.95	17.02
Enoic acid	16.89	16.95	17.02
Undecanol	17.3	17.37	17.42
Oleamide	17.3	17.37	17.42
Aspidospermidin	17.92	17.95	18.02
Pterine-6-carboxylic acid	18.25	18.31	18.4

Antibacterial activity of MGRE and CHX using disc diffusion method

The antibacterial activity of the test materials was investigated using the Disc Diffusion Method. It was found that the *S. mutans* growth inhibition zone caused by CHX was around 4 mm. However, only 2 mm growth inhibition zone was noticed around MGRE-conditioned discs. There was a statistically significant difference between them ($P \leq 0.05$), (Figure 4).

Assessment of antibacterial and antibiofilm activity of MGRE and CHX against grown *S. mutans* on titanium discs

Grown *S. mutans* on titanium discs were subjected to MGRE and CHX for 1 minute. The results showed that *S. mutans* survival in MGRE was more than CHX, while there was significantly less bacterial growth in MGRE compared to the control. The turbidity of suspended bacteria in BHIB was significantly higher in control compared to others, also, the turbidity of MGRE was Significantly higher than CHX ($P \leq 0.05$), (Figure 5 A). Regarding the antibiofilm activity, it was found that the turbidity of control, MGRE and CHX were 0.25, 0.17 and 0.03 respectively. The control was significantly higher than the others ($P \leq 0.05$) and there was a significant difference between MGRE and CHX (Figure 5 B).

The lactate production assay demonstrated that there was significantly more lactate production in control compared to others, while no significant difference between MGRE and CHX in suspension all measuring 7, 4 and 0.5 µm respectively (Figure 6 A). In addition, *S.mutans* in the biofilm produced significantly more lactate in control compared to MGRE and CHX measuring 3, 1 and 0.2 µm respectively (Figure 6 B).

Furthermore, the morphology and confluence of *S. mutans* on titanium discs were also investigated under SEM.

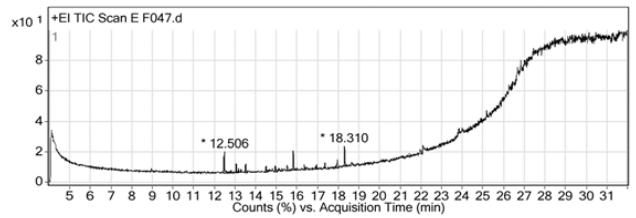

Figure 3. Percentage vs acquisition time of the detected peaks in MGRE using MC-MS.

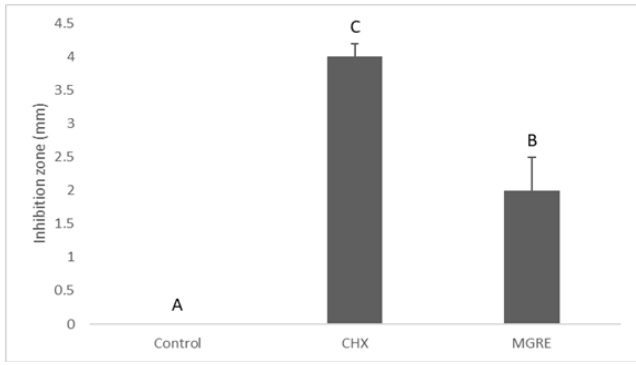

Figure 4. *S.mutans* inhibition zone around CHX or MGRE conditioned discs on agar plates. Data are mean ± S.E.M. Different letters indicate significant difference. between the variables. One-way ANOVA ($p \leq 0.05$).

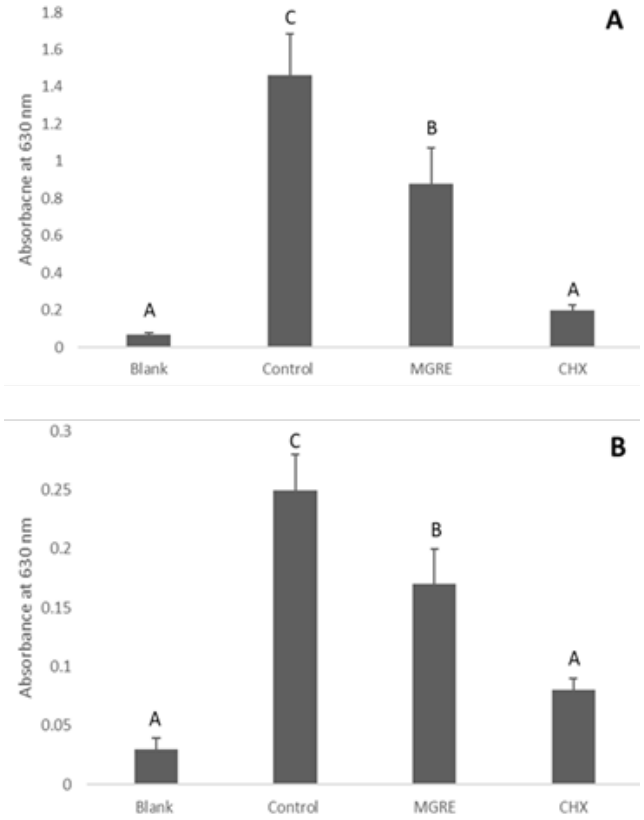

Figure 5. Assessment of antibacterial and antibiofilm activity. Data are turbidity values of suspended *S. mutans* in BHIB (A) and *S.mutans* in the biofilm (B). Data are presented as mean ± S.E.M. Different letters indicate a significant difference between the variables.

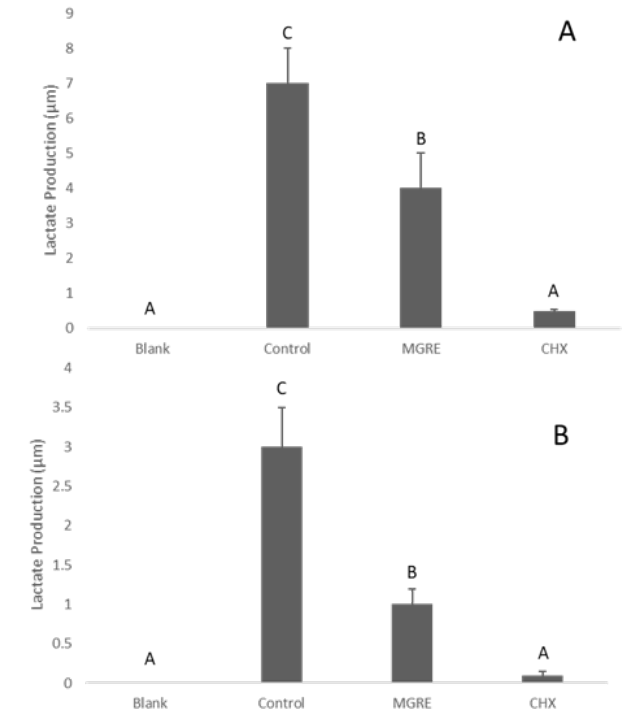

Figure 6. Lactate production by *S.mutans* in BHIB (A) and in the biofilm (B). Data are mean ± S.E.M. Different letters indicate a significant difference between the variables. One-way ANOVA ($p \leq 0.05$).

The control group showed confluent bacterial cell coverage on the substrate (Figure 7 A). However, there were no detected cells in the CHX group (Figure 7 B), and only a few cells remained adhered to the substrate after exposure to MGRE (Figure 7 C).

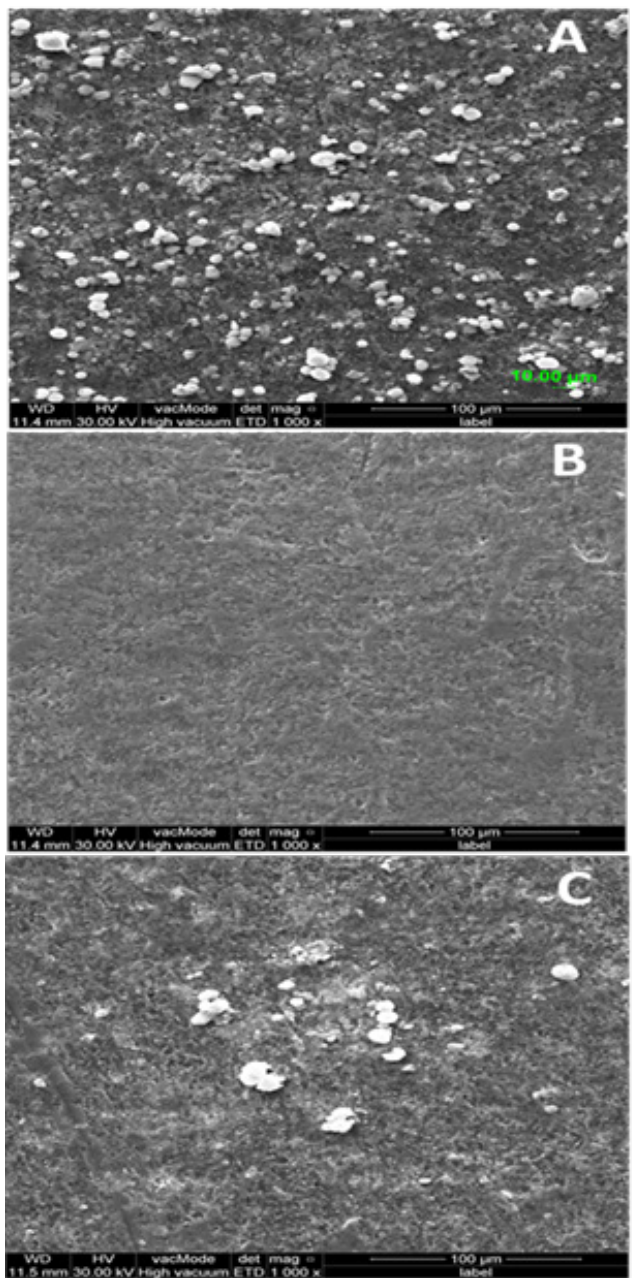

Figure 7. SEM images of *S.mutans* grown on titanium discs. (A) control, (B) CHX, (C) MGRE. Note the cell growth and confluence on the control while no cells can be detected on CHX and very limited cells on MGRE.

Discussion

The use of mouthwash to prevent peri-implant biofilm formation is grossly studied. Given the increased bacterial resistance to antibiotics, undesirable adverse effects of some antimicrobial mouthwashes currently used in dentistry as well as financial considerations in developing countries, there is a need for alternative mouthwashes that are effective, safe and economically affordable. The point is to produce a mouthwash that has an antibacterial activity against pathogenic microorganisms without interfering with the oral normal flora. This study investigated the efficacy of MGRE against *S. mutans* compared to CHX which is commonly used mouthwash in dentistry. The critical question was about the potential of MGRE as a natural (traditionally available) mouthwash that prevents peri-implant microbial colonization and subsequent infection. In this study, MGRE in an aqueous form was used, the chemical content of which was analyzed by GC-MS, the results of analysis found 12 chemical substances in the sample (Table 1). The chemical content of MGRE might change according to the chemical form of the sample, in this study, alpha penine was detected in the sample. Alpha penine is one of the substances most frequently found in MGR, for example, various studies have found that the majority of MGR essential oil is α-Pinene (26-28). The antibacterial activity of α-Pinene is well documented (29), it could be argued that the antibacterial properties of MGR are caused by the presence of α-Pinene. Moreover, α-Linolenic acid and enoic acid were also found in MGRE, similar findings have been observed by other studies (30), and both acids are known to have antibacterial properties. In addition, myrcene which is commonly found in MGR, was not detected in this study, the possible explanation for this is the fact that myrcene is not water-soluble, and the MGRE used in this study was in an aqueous form produced by boiling. Thus, myrcene was not extracted (dissolved) from the resin to the supernatant.

The microorganism used in this study was isolated from a peri-implant abutment inside the oral cavity. The peri-implant swab analysis found *S. mutans* which is considered as an early colonizer around the dental implant. *S. mutans* forms the first colony on the implant which acts as a foundation for the late colonizers (*P. gingivalis*, *P. intermedia*… etc). Thus, inhibition of *S. mutans* is critical for preventing peri-implant mucositis and peri-implantitis. The results showed that MGRE caused 2 mm inhibition zone which was significantly less than CHX which caused 4 mm growth inhibition around the CHX conditioned discs. Furthermore, antibacterial and antibiofilm activity tests of MGRE against *S. mutans* grown on titanium discs also showed similar results; it was found that MGRE caused significantly more bacterial damage compared to control, but also significantly less damage compared to CHX (Figure 5-7). This finding is supported by another study that tested the antibacterial activity of MGR against *S. mutans*, the results showed that significantly less bacteria was found in saliva after chewing mastic gum compared to those after chewing paraffin (22). Another study that investigated the efficacy of mastic chewing gum in reducing *S. mutans* concentration in the saliva of orthodontic patients also supports the existing finding; it was found in the latter that chewing mastic gum for 15 minutes caused a significant reduction of *S. mutans* in saliva (32). In addition, another study also found that MGR essential oil can cause significantly more *S. mutans* growth inhibition compared to the control (33). There are many reports (33-47) about the effect of medicinal plants in the treatment of various diseases.

The antibacterial activity of MGR against *S. mutans* is well documented. However, the efficacy of MGRE against peri-implant microbiota (bacterial biofilm on implant surface) has not been tested yet. The difference between this study the similar existing studies in literature is that in the current study, the bacteria was grown on the surface of titanium dental implant discs to mimic an *in vivo* (clinical) environment. Thus, the possible explanation for the reduced antibacterial activity of MGRE compared to CHX is that *S. mutans* were embedded in a biofilm on titanium discs. The biofilm can protect bacteria from antibacterial agents, growing bacteria on agar plates are inevitably less

protected compared to the bacterial colonies embedded in a strong biofilm on a titanium surface.

Taken together, despite the lower antibacterial activity of MGRE compared to CHX, the former still has the potential to be used as a mouthwash to prevent peri-implant mucositis as it significantly prevented *S. mutans* growth compared to the negative control. MGRE is a natural product and has numerous diverse pharmaceutical and biomedical properties that make it a good substitute for the currently used chemical mouthwashes. Using MGRE as a mouthwash can reduce the side effects of currently used mouthwashes, provide adjunctive health benefits for the patient and be financially more affordable than the existing chemical mouthwashes. Further studies, especially clinical trials are needed to investigate the effect of MGRE mouthwash in reducing the incidence of peri-implant mucositis.

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Conflict of interest
The author declares no conflict of interest.

References

1. Albrektsson T, editor Consensus report of session IV. Proceedings of the first European workshop on periodontology; 1994: Quintessence Int 1994:365-369.
2. Roos-Jansåker AM, Renvert H, Lindahl C, Renvert S. Nine-to fourteen-year follow-up of implant treatment. Part III: factors associated with peri-implant lesions. J Clin Periodontol 2006;33(4):296-301.
3. Heitz-Mayfield LJ, Salvi GE. Peri-implant mucositis. J Clin Periodontol 2018;45:237-245.
4. Salvi GE, Aglietta M, Eick S, Sculean A, Lang NP, Ramseier CA. Reversibility of experimental peri-implant mucositis compared with experimental gingivitis in humans. Clin Oral Implants Res 2012;23(2):182-190.
5. Ramanauskaite A, Fretwurst T, Schwarz F. Efficacy of alternative or adjunctive measures to conventional non-surgical and surgical treatment of peri-implant mucositis and peri-implantitis: A systematic review and meta-analysis. Int J Implant Dent 2021;7(1):1-61.
6. Ji YJ, et al. Efect of glycine powder air-polishing as an adjunct in the treatment of peri-implant mucositis: a pilot clinical trial. Clin Oral Implants Res 2014;25(6):683–9
7. Wohlfahrt JC, Aass AM, Koldsland OC. Treatment of peri-implant mucositis with a chitosan brush—a pilot randomized clinical trial. Int J Dent Hyg 2019;17(2):170–6.
8. Al Rifaiy MQ, et al. Efectiveness of adjunctive antimicrobial photodynamic therapy in reducing peri-implant infammatory response in individuals vaping electronic cigarettes: a randomized controlled clinical trial. Photodiagnosis Photodyn Ther 2018;22:132–6.
9. Deeb MA, et al. Clinical and microbiological outcomes of photodynamic and systemic antimicrobial therapy in smokers with peri-implant infammation. Photodiagnosis Photodyn Ther 2020;29:101587.
10. Aimetti M, et al. Adjunctive efcacy of diode laser in the treatment of peri-implant mucositis with mechanical therapy: a randomized clinical trial. Clin Oral Implants Res 2019;30(5):429–38.
11. Mariani GM, et al. One-year clinical outcomes following non-surgical treatment of peri-implant mucositis with adjunctive diode laser application. Minerva Stomatol 2020;69(5):269–77.
12. Thöne-Mühling M, et al. Comparison of two full-mouth approaches in the treatment of peri-implant mucositis: a pilot study. Clin Oral Implants Res 2010;21(5):504–12. 34.
13. Menezes KM, et al. Efcacy of 0.12% chlorhexidine gluconate for non-surgical treatment of peri-implant mucositis. J Periodontol 2016;87(11):1305–13.
14. Iorio-Siciliano V, et al. Anti-infective therapy of peri-implant mucositis with adjunctive delivery of a sodium hypochlorite gel: a 6-month randomized triple-blind controlled clinical trial. Clin Oral Investig 2020;24(6):1971–9.
15. Koban I, Holtfreter B, Hübner NO, Matthes R, Sietmann R, Kindel E, Weltmann KD, Welk A, Kramer A, Kocher T. Antimicrobial efficacy of non-thermal plasma in comparison to chlorhexidine against dental biofilms on titanium discs in vitro–proof of principle experiment. J Clin Periodontol 2011;38(10):956-65.
16. Boakye YD, Osafo N, Danquah CA, Adu F, Agyare C. Antimicrobial agents: Antibacterial agents, anti-biofilm agents, antibacterial natural compounds, and antibacterial chemicals. Antimicrobials, Antibiotic Resistance, Antibiofilm Strategies and Activity Methods 2019;13:75.
17. Hobson C, Chan AN, Wright GD. The antibiotic resistome: a guide for the discovery of natural products as antimicrobial agents. Chem Rev 2021;121(6):3464-94.
18. Alqahtani A, Mohamed AA, Ahmad I, Alraey Y, Asiri M, Kopel J, Alshahrani MM. Pomegranate peel extract is an effective agent against MDR bacteria. Cell Mol Biol 2023;69(5):6-11.
19. Gupta D, Nayan S, Tippanawar HK, Patil GI, Jain A, Momin RK, Gupta RK. Are herbal mouthwash efficacious over chlorhexidine on the dental plaque?. Pharmacognosy Res 2015;7(3):277.
20. Rahman HS. Phytochemical analysis and antioxidant and anticancer activities of mastic gum resin from Pistacia atlantica subspecies kurdica. Onco Targets Ther 2018; 6:4559-72.
21. Paraschos S, Magiatis P, Mitakou S, Petraki K, Kalliaropoulos A, Maragkoudakis P, Mentis A, Sgouras D, Skaltsounis AL. In vitro and in vivo activities of Chios mastic gum extracts and constituents against Helicobacter pylori. Antimicrob. Agents chemother 2007;51(2):551-9.
22. Aksoy A, Duran N, Koksal F. In vitro and in vivo antimicrobial effects of mastic chewing gum against Streptococcus mutans and mutans streptococci. Arch Oral Biol 2006 1;51(6):476-81.
23. Salaie, R., 2018. Nano Enhanced Surface Modification of Titanium Dental Implants for Improving Osseointegration and Biocompatibility (Doctoral dissertation, University of Plymouth).
24. Salaie, R.N., Besinis, A., Le, H., Tredwin, C. and Handy, R.D. The biocompatibility of silver and nanohydroxyapatite coatings on titanium dental implants with human primary osteoblast cells. Mater Sci Eng: C 2020; 107:110210.
25. Salaie, R.N., Hassan, P.A., Meran, Z.D. and Hamad, S.A. Antibacterial Activity of Dissolved Silver Fractions Released from Silver-Coated Titanium Dental Implant Abutments: A Study on Streptococcus mutans Biofilm Formation. Antibiotics 2023;12(7): 1097-1108
26. Gortzi, O.; Athanasiadis, V.; Lalas, S.; Chinou, I.; Tsaknis, J. Study of antioxidant and antimicrobial activity of Chios mastic gum fractions (neutral, acidic) before and after encapsulation in liposomes. J Food Process Technol 2014;5(1):1-5.
27. Paraschos, S.; Magiatis, P.; Gikas, E.; Smyrnioudis, I.; Skaltsounis, A.L. Quality profile determination of Chios mastic gum essential oil and detection of adulteration in mastic oil products with the application of chiral and non-chiral GC–MS analysis. Fitoterapia 2016;14:12–17
28. Rigling, M.; Fraatz, M.A.; Stefan Troegel, S.; Jinyuan Sun, J.;

Holger Zorn, H.; Zhang, Y. Aroma investigation of Chios mastic gum (Pistacia lentiscus variety chia) using headspace gas chromatography combined with olfactory detection and chiral analysis. J Agric Food Chem 2019;67:13420–13429.
29. De Sousa Eduardo, L.; Farias TC.; Ferreira SB.; Ferreira PB.; Lima ZN.; Ferreira SB. Antibacterial activity and time-kill kinetics of positive enantiomer of α-pinene against strains of Staphylococcus aureus and Escherichia coli. Curr Top Med Chem 2018;18(11):917-924.
30. Tabanca N.; Nalbantsoy A.; Kendra PE.; Demirci F.; Demirci B. Chemical characterization and biological activity of the mastic gum essential oils of Pistacia lentiscus var. chia from Turkey. Molecules 2020;25(9):2136-2155.
31. Aksoy A, Duran N, Toroglu S, Koksal F. Short-term effect of mastic gum on salivary concentrations of cariogenic bacteria in orthodontic patients. Angle Orthod 2007 Jan 1;77(1):124-8.
32. Hosseini F, Adlgostar A, Sharifnia F. Antibacterial activity of Pistacia atlantica extracts on Streptococcus mutans biofilm. Int Res J Biological Sci 2013;2(2):1-7.
33. Noori A, Zebarjadi A. Introduction of Chia (Salvia hispanica L.) as an Important Oil-Medicinal Plant. Agrotech Ind Crops 2022; 2(3): 104-116. doi: 10.22126/atic.2022.8010.1060.
34. Chaghakaboodi Z, Nasiri J, Farahani S. Fumigation Toxicity of the Essential Oils of Ferula persica against Tribolium castaneum and Ephestia kuehniella. Agrotech Ind Crops 2022; 2(3): 123-130. doi: 10.22126/atic.2022.8344.1068.
35. Ganjali S, Khajeh H, Gholami Z, Jomeh-ghasemabadi Z, Fazeli-Nasab B. Evaluation of Dormancy Failure Datura stramonium Plant Seeds under the Influence of Different Treatments. Agrotech Ind Crops 2022; 2(1): 32-41. doi: 10.22126/atic.2022.7656.1049.
36. Ghamarnia H, Palash M, Dousti B. Camelina Zoning for Different Climate Conditions in Kurdistan Province. Agrotech Ind Crops 2022; 2(1): 49-56. doi: 10.22126/atic.2022.7903.1056.
37. Ghamarnia H, Mousabeygi F, Rezvani SV. Water Requirement, Crop Coefficients of Peppermint (Mentha piperita L.) and Realizing of SIMDualKc Model. Agrotech Ind Crops 2021; 1(3): 110-121. doi: 10.22126/atic.2021.6791.1019.
38. Aryafar S, Sirousmehr A, Najafi S. The Impact of Compost on Seed Yield and Essential Oil of Black Cumin under Drought Stress Conditions. Agrotech Ind Crops 2021; 1(3): 139-148. doi: 10.22126/atic.2021.7184.1026.
39. Teimoori, N., Ghobadi, M., Kahrizi, D. Improving the Growth Characteristics and Grain Production of Camelina (Camelina sativa L.) under Salinity Stress by Silicon Foliar Application. Agrotech Ind Crops 2023; 3(1): 1-13. doi: 10.22126/atic.2023.8681.1081.
40. Norani M, Crawford A, Aliahmadi A, Ayyari M. Evaluation of Tussilago farfara L. Smoke by GC/MS: A Phytochemical Approach to a Traditional Medicine. Agrotech Ind Crops 2023; 3(1): 14-22. doi: 10.22126/atic.2023.8818.1083.
41. Kakaei M, Rehman FU. Cryopreservation Procedure Technique in Medicinal Plants. Agrotech Ind Crops 2023; 3(1): 23-29. doi: 10.22126/atic.2023.8978.1088.
42. Rostami Ahmadvandi H, Alavi SM, Jabbari H, Jamshid Moghaddam M. Quantitative and Qualitative Evaluation of Safflower (Carthamus tinctorius L.) Mutants in Comparison with Commercially Released Cultivars in Iran. Agrotech Ind Crops 2023; 3(1): 38-43. doi: 10.22126/atic.2023.9039.1093
43. Morovati Z, Karimzadeh G, Naghavi MR, Rashidi Monfared S. Essential Oil Profile of Areal Part of Datura stramonium L. and D. innoxia from Iran. Agrotech Ind Crops 2023; 3(2): 53-59. doi: 10.22126/atic.2023.9064.1094
44. Ghamarnia H, Basiri M, Ghobadi M, Palash M. Performance of Peppermint (Mentha piperita L.) in Different Water Deficit and Salinity Management. Agrotech Ind Crops 2023; 3(2): 84-95. doi: 10.22126/atic.2023.8839.1085
45. Zaman GS, Alshahrani MY, Barua P, Aladel A, Zaman FA, Ahmad I, Khan MI, Snoussi M, Saeed M. Screening of the antioxidant and antibacterial effects of extracted essential oils from Thunbergia coccinea, Acacia polyacantha, Polygonum micrpcephallum, Abies spectabilis and Clerodendrum colebrookianum. Cell Mol Biol (Noisy-le-grand). 2022 Jan. 2;67(4):56-67. Available from: https://www.cellmolbiol.org/index.php/CMB/article/view/4037.
46. Muhialdin AJ, Alamri ZZ, M. Hussein A, Faraj RK, Taha ZB, Hussein MM, Mojarradgandoukmolla S. Gastro-Protective and Therapeutic Effect of Punica granatum against Stomach Ulcer Caused by Helicobacter Pylori: Effect of Punica granatum against Stomach Ulcer . Cell Mol Biol (Noisy-le-grand). 2023 Jan. 69(1):48-53. Available from: https://www.cellmolbiol.org/index.php/CMB/article/view/4646.
47. Sasani S, Rashidi Monfared S, Mirzaei AR. Identification of some Echinophora platyloba miRNAs using computational methods and the effect of these miRNAs in the expression of TLN2 and ZNF521 genes in different human body organs. Cell Mol Biomed Rep 2024;4(1): 43-53. doi: 10.55705/cmbr.2023.386145.1100.