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RESEARCH ARTICLE **OPEN ACCESS**

The Effects of *Hypericum perforatum* Nanoemulsion on *Streptococcus Mutans* Biofilm

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Abstract:

Background: Dental caries, a multifactorial tooth-related disease, is significantly influenced by microorganisms, like *Streptococcus mutans*. This bacterium causes dental caries and destruction by forming a biofilm on the tooth surface. This study aimed to prepare *Hypericum perforatum* nanoemulsion and evaluate its anti-biofilm effects against *Streptococcus mutans* bacteria.

Methods: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Minimum Biofilm Inhibitory Concentration (MBIC) were used to assess the antimicrobial and anti-biofilm properties of the nanoemulsion.

Results: The results showed that the *Hypericum perforatum* nanoemulsion had less MIC, MBC, and MBIC than the free oil form (p=0.03, p=0.03, and p=0.02, respectively). The results also showed that the *Hypericum perforatum* nanoemulsion had less MIC and MBC than amikacin as a positive group (p=0.04 and p=0.04, respectively); however, the MBIC of nanoemulsion and amikacin was similar (p=0.07).

Conclusion: The studied nanoemulsion can be used as a potent and new material for preventing dental caries.

Keywords: Dental caries, *Hypericum perforatum*, Nanoemulsion, *Streptococcus mutans*, Biofilm, Antimicrobial.

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1. INTRODUCTION

Streptococcus mutans is known to be involved in tooth decay [1, 2]. As a Gram-positive bacterium, it achieves adhesion capability by synthesizing glucans. It collaborates with other types of bacteria to create a biofilm on teeth [3], which ultimately causes tooth decay. A biofilm is an aggregation of bacterial cells that firmly adheres to the surface of a tooth. Bacteria are enclosed in a self-secreted extracellular matrix. This matrix is a protective shield, guarding bacterial cells against harmful environmental factors, antibiotics, and the host's immune defenses [3, 4]. *Streptococcus mutans*, through carbohydrate fermentation and acid production, lowers the pH. As a result, the acidic environment enhances the solubility and demineralization of dental tissue, ultimately leading to tooth decay [5, 6].

To prevent tooth decay, various substances can be used to destroy or inactivate the bacteria responsible for decay [7]. The preventive agents include antibiotics, fluoride compounds, and chlorhexidine [1, 5]. These anti-

decay agents are produced and available in different forms, such as mouthwash, gel, or toothpaste. While these products offer benefits in fighting cavities (anti-caries effects), most of them contain ingredients that can lead to unintended consequences. These potential side effects include disrupting the natural balance of bacteria in the mouth and gut (oral and digestive flora), digestive issues, like diarrhea and vomiting, development of bacterial resistance, and even discoloration of teeth [8, 9]. Indeed, according to scientific literature, some ingredients of these products, such as fluoride and sodium dodecyl sulfate, remain on the teeth and the oral environment and enter the digestive system through swallowing [3, 5, 8, 10].

Natural herbal products may show fewer risks and side effects compared to chemical products [11, 12]. Some effective ingredients against decay-causing micro-organisms include ginger, clove oil, neem oil, garlic essence, and tea tree oil. These can be incorporated into mouthwashes, toothpaste, and topical gels for maintaining oral health [10].

Hypericum perforatum is one of the plants used in traditional medicine [13]. It has numerous uses, including in the treatment of bacterial infections, Acquired Immunodeficiency Syndrome (AIDS), malignancies, burns, and digestive problems [14]. This plant extract appears promising as a natural ingredient in dental care products. Researchers suggest that it has antibacterial properties effective against several bacteria commonly found in the mouth, including *Lactobacillus plantarum*, *Streptococcus mutans*, *Streptococcus sobrinus*, and *Enterococcus faecalis* [7]. Bagheri *et al*. explored that *Hypericum perforatum* inhibited the growth of *Streptococcus mutans*, *Escherichia coli*, and *Enterococcus faecalis*, and prevented them from forming biofilm [15].

The introduction of nanotechnology techniques in medical research has led to the use of nanomaterials in the diagnosis, treatment, and control of biological systems [14]. Nanoemulsions are non-equilibrium systems characterized by droplet sizes in the nanometer range (typically 20 to 200 nm) [16, 17]. These systems offer an excellent option for creating nanoscale versions of plant oils. Since plant ingredients are insoluble in water, their bioavailability and effectiveness in the body are limited. However, by preparing nanoemulsions of these oils, their solubility and bioavailability can be enhanced, leading to more effective outcomes [18].

Researchers have explored the impact of nanoemulsions derived from various vegetable oils on *Streptococcus mutans* biofilm, a pivotal contributor to dental caries, by preparing a soybean oil nano-emulsion with an MIC of 250 µl [18], a cinnamon nano-emulsion that has emerged as the most potent agent against biofilm [19], and curcumin nano-emulsion that has exhibited significant antibacterial and anti-biofilm properties [20].

Therefore, this study aimed to explore the impact of the nanoemulsion of *Hypericum perforatum* on the biofilm of *Streptococcus mutans*, which serves as the primary pathogen in dental caries.

2. METHODS AND MATERIALS

The nanoemulsion was prepared in our previous study [21]. Briefly, tween 80 surfactant (2% w/w, Sigma Ultra, low peroxide) was gradually added to *Hypericum perforatum* oil (4% w/w, Barij Esans Co., Tehran, Iran), and then the distilled water (Takrou, Tehran, Iran) was added slowly to the mixture, and the mixture was stirred for 10 minutes at 1000 rpm. Then, the formed emulsion was transferred to an ultrasonic device (at 17000 rpm) to finalize the preparation of the nanoemulsion (Tomey, Erlangen, Germany, 400 W, 20). The prepared nanoemulsion was then characterized using conventional methods. The prepared nanoemulsion demonstrated proper physicochemical properties with no cytotoxicity against human Gingival Fibroblasts (hGFs) [21].

2.1. Sample Size

The results of three repetitions were averaged and expressed as descriptive statistics (n=12 for each test). The study included 4 groups as follows:

- Group 1: *Hypericum perforatum* nanoemulsion
- Group 2: *Hypericum perforatum* free oil
- Group 3: negative control: this group served as a baseline and only contained the culture medium typically used to grow bacteria. It did not include any antibiotics or the nano-emulsion.
- Group 4: positive control: this group included the amikacin antibiotic, known to be effective against bacteria.

2.2. Inclusion Criteria

The inclusion criteria employed were the use of *Streptococcus mutans* ATCC25175 standard strain (Pastor Institute, Tehran, Iran), well-defined and validated *Hypericum perforatum* oil (Barij Esans Co, Tehran, Iran), and the standard antimicrobial tests (MIC, MBC, MBIC).

2.3. Exclusion Criteria

Samples with any contamination were excluded.

2.4. Evaluation of Antimicrobial and Anti-biofilm Effects

Hypericum perforatum nanoemulsion and *Hypericum perforatum* free oil were serially diluted 1:2 (10 times) in Mueller Hinton broth. A suspension of *Streptococcus mutans* (10^6 CFU/mL) was added to the serially diluted tubes. Following 20 h of incubated exposure (37°C), the tubes were inspected visually to determine the turbidity. The concentration with turbidity was recorded as the MIC value. For MBC determination, the measured quantities were transferred from the dilution suspension representing the MIC and from the two dilution suspensions preceding the MIC dilution to plates of solid growth medium, which were then incubated for 20 h at 37°C. Following incubation, the plates were examined for the growth of the microorganism. The concentration that produced no growth was recorded as the MBC.

Table 1. MIC and MBC values of the studied groups.

MIC (µl)				MBC (µl)			
Nanoemulsion	Free Oil	Positive Control	Negative Control	Nanoemulsion	Free Oil	Positive Control	Negative Control
0.041±0.01	0.6±0.23	0.08±0.02	0	0.08±0.02	1.1±1.11	0.16±0.11	0

Table 2. MBIC of the studied groups.

MBIC (µl)			
Nanoemulsion	Oil	Positive Control	Negative Control
0.08±0.11	0.5±0	0.08±0.02	0

The Minimum Biofilm Inhibitory Concentration (MBIC) was determined to assess the anti-biofilm properties. MBIC demonstrated the lowest concentration of antimicrobial substances, resulting in a difference of less than 10% compared to the standard Optical Density (OD).

A suspension of *Streptococcus mutans* that matched the density of the 0.5 McFarland standard was prepared. The bacterial suspension was placed in a special micro-plate. Each well contained a growth medium called Mueller Hinton broth. The plate was incubated for 20 h at 37°C, which is a favorable temperature for bacterial growth. After incubation, the contents (serially diluted 1:2, 10 times) were removed from each well and washed with a sterile solution. Then, various diluted concentrations of the extract were added. The treated wells were incubated again for 20 h at 37°C. Once more, the contents were removed, and the wells were washed. Fresh culture medium (without any antimicrobials) was added to each well. The spectrophotometer was used to measure the OD of the cultures at a specific wavelength (650 nm) before and after an additional 6 h of incubation. The OD showed the culture's cloudiness, indicating bacterial growth.

2.5. Statistical Analysis

The data were summarized using descriptive statistics. A normality test (Shapiro-Wilk) was conducted to determine normality. One-way ANOVA was used to compare the data. GraphPad software was used to analyze the data. A *p*-value less than 0.05 was considered a statistically significant level.

3. RESULTS

The results showed that the *Hypericum perforatum* nanoemulsion had less MIC, MBC, and MBIC values than the free oil form (p=0.03, p=0.03, and p=0.02, respectively). The results also showed that the *Hypericum perforatum* nanoemulsion had less MIC and MBC values than the positive group (p=0.04 and p=0.04, respectively); however, the MBIC of the two groups was similar (p=0.07). Tables **1** and **2** summarize the results.

4. DISCUSSION

The studies have shown different forms of *H. perforatum* extracts to have different antibacterial properties against both Gram-positive and Gram-negative bacteria [22, 23]. The secondary metabolites of *H. perforatum*, mainly n-hexane and ethyl acetate, have been revealed to exhibit better antibacterial activity against a considerable number of Multidrug-resistant (MDR) clinical isolates of Gram-positive bacteria compared to broad-spectrum antibiotics, as defined by susceptibility breakpoints [23]. An additional study found that the essential oils derived from *H. perforatum* contained a substantial quantity of aromatic polyketides, such as hypericin, indicating anti-fungal and anti-yeast characteristics against pathogenic microorganisms [24, 25]. According to Khademnejad *et al.*, the hypericin present in this plant can remove acid-producing strains, like *Streptococcus mutans* [26]. Based on a recent study, all different forms of *H. perforatum* extracts consisting of oils and organic extracts demonstrated some degree of ability to prevent biofilm formation. That study also determined antibacterial activities across all strains of *S. aureus* and demonstrated that the aqueous extract of flowering aerial parts and methanol extract of flowering aerial parts exhibited growth inhibition properties [27].

Due to the main role of *Streptococcus mutans* in dental caries, this study offered promising insights into the antimicrobial capacity of *Hypericum perforatum* nanoemulsion against *Streptococcus mutans*. The results showed that the *Hypericum perforatum* nanoemulsion exhibited better antimicrobial and antibiofilm functions than the free oil form. It also exerted better antimicrobial effects than the positive group; however, the antibiofilm effects of the two groups were similar.

In our study, the *Hypericum perforatum* nanoemulsion demonstrated higher antimicrobial effects, as evidenced by lower MIC, MBC, and MBIC values compared to the free oil form (p=0.03, p=0.03, and p=0.02, respectively). These findings were found to be consistent with the work of Bagheri *et al.*, who showed that *H. perforatum* oil inhibited bacterial growth and prevented biofilm formation [15]. However, the enhanced efficacy of the nanoemulsion in our study aligned with the research performed by Li *et al.*, which highlighted the superior antimicrobial activity of nanoemulsions compared to conventional formulations due to better stability and bioavailability [28].

Interestingly, while Horváth *et al.* [19] reported higher MIC values for essential oil nanoemulsions against

Streptococcus mutans, our study found lower MIC and MBC values for *H. perforatum* nanoemulsion compared to both the free oil form and the positive control (p=0.04 and p=0.04, respectively). This suggests that our nanoemulsion formulation may have distinct physicochemical properties, contributing to its enhanced antimicrobial action. However, similar to the results of Horváth *et al*., the antibiofilm activity (MBIC) of the nanoemulsion and the positive control group in our study was statistically similar (p=0.07), indicating that while nanoemulsions may improve antimicrobial potency, their antibiofilm capabilities might not always outperform conventional treatments.

Moreover, Khademnejad *et al*. [26] reported the role of hypericin in removing *Streptococcus mutans*, which may explain the similar MBIC values observed between the nanoemulsion and positive control groups in our study. This suggests hypericin, along with other bioactive compounds, to play a key role in the biofilm inhibition observed.

4.1. Strengths and Limitations

One of the strengths of our study is the demonstrated superiority of *Hypericum perforatum* nanoemulsion over the free oil form, particularly in terms of MIC and MBC, aligning with prior research suggesting that nanoemulsions offer enhanced antimicrobial effects [9, 20]. The lower concentrations required in our study, compared to earlier studies, provide promise for the development of more efficient and potentially safer formulations, which could be valuable for clinical applications, especially in dental care for *Streptococcus mutans*.

However, a notable limitation of our study is its *in vitro* nature, which does not fully reflect *in vivo* conditions. Additionally, while the antimicrobial effects of the nanoemulsion were superior, the similar MBIC values between the nanoemulsion and the positive control group indicated that further research is needed to improve its antibiofilm capacity. This is consistent with studies, like that of Horváth *et al*. [12], where nanoemulsions demonstrated variable biofilm inhibition depending on the formulation. Future studies should focus on optimizing the nanoemulsion's composition to enhance its antibiofilm effects and verify these findings in clinical settings.

Moreover, it remains unclear whether the enhanced effects observed in the study were solely due to the nanoemulsion formulation or if specific bioactive compounds, like hypericin, were responsible. As previous studies have highlighted the role of different forms of *H. perforatum* extracts in varying antimicrobial activity [16, 19], further investigation of the exact mechanism of action is required to fully understand and exploit the therapeutic potential of *H. perforatum* nanoemulsions.

CONCLUSION

The prepared nanoemulsion showed the lowest MIC, MBC, and MBIC values among the studied groups, indicating its antibacterial and antibiofilm effectiveness. Therefore, we suggest conducting more studies in order to examine the chance of using this material as an alternative to antibiotics owing to their restrictions.

AUTHORS' CONTRIBUTION

A.Z., Y.P., B.K.: Data curation; S.K.: Data analysis or interpretation; S.G.: Writing, review, and editing; S.M.D.: Writing of the original draft. All authors have reviewed the results and approved the final version of the manuscript.

LIST OF ABBREVIATIONS

MIC = Minimum Inhibitory Concentration

MBC = Minimum Bactericidal Concentration

MBIC = Minimum Biofilm Inhibitory Concentration

ETHICAL STATEMENT

This study recived the ethics code from Ethics committe of Tabriz University of Medical Sciences, Iran (IR.TBZMED.VCR.REC.1402.017).

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study are included in this published article.

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CONFLICT OF INTEREST

The author(s) declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Namburu JR, Rajendra Sanosh AB, Poosarla CS, Manthapuri S, Pinnaka M, Baddam VRR. *Streptococcus M utans*- specific antimicrobial peptide C16G2-mediated caries prevention: A review. Front Dent 2022; 19: 17. PMID: 36458273

[2] Hassan WL, Erdeni AA. Isolation and diagnosis of *Streptococcus mutans* and *Streptococcus sorbinus* from patients with tooth decay and gingivitis. Tikrit J of Pure Science 2024; 29(1): 37-48.

[3] Zhang Z, Yang Y, Sun Q, Zeng W, Li Y. Inhibition of biofilm formation and virulence factors of cariogenic oral pathogen *Streptococcus mutans* by shikimic acid. Microbiol Spectr 2022; 10(4): e01199-22. http://dx.doi.org/10.1128/spectrum.01199-22 PMID: 35880891

[4] Magana M, Sereti C, Ioannidis A, *et al*. Options and limitations in clinical investigation of bacterial biofilms. Clin Microbiol Rev 2018; 31(3): e00084-16. http://dx.doi.org/10.1128/CMR.00084-16 PMID: 29618576

[5] Carvalho ÊS, Ayres VFS, Oliveira MR, *et al*. Anticariogenic activity of three essential oils from Brazilian piperaceae. Pharmaceuticals

2022; 15(8): 972.
http://dx.doi.org/10.3390/ph15080972 PMID: 36015120

[6] Gad El-Rab SMF, Ashour AA, Basha S, Alyamani AA, Felemban NH, Enan ET. Well-orientation strategy biosynthesis of cefuroxime-silver nanoantibiotic for reinforced biodentine™ and its dental application against *Streptococcus mutans*. Molecules 2021; 26(22): 6832.
http://dx.doi.org/10.3390/molecules26226832 PMID: 34833924

[7] Süntar I, Oyardı O, Akkol EK, Ozçelik B. Antimicrobial effect of the extracts from *Hypericum perforatum* against oral bacteria and biofilm formation. Pharm Biol 2016; 54(6): 1065-70.
http://dx.doi.org/10.3109/13880209.2015.1102948 PMID: 26510970

[8] Desu HR, Narang AS, Kumar V, Thoma LA, Mahato RI. Liquid dosage forms. Pharmaceutics. Elsevier 2024; pp. 271-318.

[9] Fejerskov O, Cury J, Tenuta L. Fluorides in caries control. 2015; 245-76.

[10] Kanth MR, Prakash AR, Sreenath G, Reddy VS, Huldah S. Efficacy of specific plant products on microorganisms causing dental caries. J Clin Diagn Res 2016; 10(12): ZM01-3.
http://dx.doi.org/10.7860/JCDR/2016/19772.9025 PMID: 28209019

[11] Ichim MC, Scotti F. Quality evaluation of commercial herbal products using chemical methods. Crit Rev Food Sci Nutr 2024; 64(13): 4219-39.

[12] Bent S. Herbal medicine in the United States: review of efficacy, safety, and regulation: Grand rounds at University of California, San Francisco Medical Center. J Gen Intern Med 2008; 23(6): 854-9.

[13] Farasati Far B, Gouranmohit G, Naimi-jamal MR, Neysani E, El-Nashar HA, El-Shazly M. The potential role of *Hypericum perforatum* in wound healing: A literature review on the phytochemicals, pharmacological approaches, and mechanistic perspectives. Phytother Res 2024; 38(7): 3271-95.
http://dx.doi.org/10.1002/ptr.8204

[14] Alahmad A, Al-Zereini WA, Hijazin TJ, *et al*. Green synthesis of silver nanoparticles using *Hypericum perforatum* L. aqueous extract with the evaluation of its antibacterial activity against clinical and food pathogens. Pharmaceutics 2022; 14(5): 1104.
http://dx.doi.org/10.3390/pharmaceutics14051104 PMID: 35631691

[15] Bagheri R, Bohlouli S, Maleki Dizaj S, Shahi S, Memar MY, Salatin S. The antimicrobial and anti-biofilm effects of *Hypericum perforatum* oil on common pathogens of periodontitis: An *in vitro* study. Clin Pract 2022; 12(6): 1009-19.
http://dx.doi.org/10.3390/clinpract12060104 PMID: 36547112

[16] Shah P, Bhalodia D, Shelat P. Nanoemulsion: A pharmaceutical review. Sys Rev Pharm 2010; 1(1): 24-32.
http://dx.doi.org/10.4103/0975-8453.59509

[17] Mushtaq A, Wani SM, Malik A, Gull A, Ramniwas S, Nayik GA. Recent insights into nanoemulsions: Their preparation, properties and applications. Food Chem X 2023 Apr 20;18: 2023; 18: 100684.

[18] Ramalingam K, Amaechi BT, Ralph RH, Lee VA. Antimicrobial activity of nanoemulsion on cariogenic planktonic and biofilm organisms. Arch Oral Biol 2012; 57(1): 15-22.
http://dx.doi.org/10.1016/j.archoralbio.2011.07.001 PMID: 21807359

[19] Horváth B, Balázs VL, Varga A, *et al*. Preparation, characterisation and microbiological examination of Pickering nano-emulsions containing essential oils, and their effect on *Streptococcus mutans* biofilm treatment. Sci Rep 2019; 9(1): 16611.
http://dx.doi.org/10.1038/s41598-019-52998-6 PMID: 31719559

[20] Cho MY, Kang SM, Lee ES, Kim BI. Antimicrobial activity of *Curcuma xanthorrhiza* nanoemulsions on *Streptococcus mutans* biofilms. Biofouling 2020; 36(7): 825-33.
http://dx.doi.org/10.1080/08927014.2020.1823376 PMID: 32972257

[21] Gargari FK, Yasamineh N, Atashi YG, Dizaj SM, Sharifi SJTODJ. Stability and cytotoxicity assessments of *Hypericum perforatum* nanoemulsion in preventing denture-related stomatitis. The Open Dentistry 2024; 18(1): e18742106308988.
http://dx.doi.org/10.2174/0118742106308988240525165932

[22] Barbagallo C, Chisari G. Antimicrobial activity of three Hypericum species. Fitoterapia 1987; 58(3): 175-7.

[23] Kisa O, Oksuz L, Servi H, Aysal AI. Antibacterial activity of *Hypericum perforatum* L. (St. John's wort) extracts against Gram-positive bacteria and characterisation of its secondary metabolites. Nat Prod Res 2023; 1-8.
http://dx.doi.org/10.1080/14786419.2023.2291702 PMID: 38093524

[24] Sytar O, Švedienė J, Ložienė K, Paškevičius A, Kosyan A, Taran N. Antifungal properties of hypericin, hypericin tetrasulphonic acid and fagopyrin on pathogenic fungi and spoilage yeasts. Pharm Biol 2016; 54(12): 3121-5.
http://dx.doi.org/10.1080/13880209.2016.1211716 PMID: 27564138

[25] Wu S. Specialized metabolism in St John's wort. Curr Opin Plant Biol 2024; 82(102625)

[26] Nezhad SK, Zenouz AT, Aghazadeh M, Kafil HSJC, Biology M. Strong antimicrobial activity of *Hypericum perforatum* L. against oral isolates of *Lactobacillus* spp. Cell Mol Biol 2017; 63(11): 58-62.

[27] Lyles JT, Kim A, Nelson K, *et al*. The chemical and antibacterial evaluation of St. John's wort oil macerates used in Kosovar Traditional Medicine. Front Microbiol 2017; 8: 1639.
http://dx.doi.org/10.3389/fmicb.2017.01639 PMID: 28943862

[28] Li G, Zhang Z, Liu H, Hu L. Nanoemulsion-based delivery approach for nutraceuticals: Fabrication, application, characterization, biological fate, potential toxicity and future trends. Food Funct 2021; 12(5): 1933-53.