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RESEARCH ARTICLE **OPEN ACCESS**

The Antifungal Effects of *Hypericum perforatum* Nanoemulsion against *Candida albicans*

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Abstract:

Background: Oral stomatitis is one of the common infections related to dentures (complete or partial), which is seen in the form of diffuse inflammation, mainly with *Candida albicans*. The aim of the present study was to investigate the antifungal effects of *Hypericum perforatum* nanoemulsion against *Candida albicans*.

Materials and Methods: The microdilution technique has been used in order to determine the antifungal effects of *Hypericum perforatum* nanoemulsion. The study has involved four groups: *Hypericum perforatum* oil, *Hypericum perforatum* nanoemulsion, distilled water as a negative control, and nystatin as a positive control. The first concentration of nanoemulsion in which no clear fungal growth was observed in the culture medium has been considered as the Minimum Inhibitory Concentration (MIC) and the first concentration in which the fungal growth was completely stopped and no fungal growth was observed in the culture medium has been considered as the Minimum Fungicide Concentration (MFC). One-way Analysis of Variance (ANOVA) has been employed to compare the results between the groups. Tukey's post-hoc test has been used to make comparisons between the groups. The significance level has been considered at less than 0.05.

Results: Both *Hypericum perforatum* oil and *Hypericum perforatum* nanoemulsion have been found to exhibit good antifungal activity against *Candida albicans*. The MIC and the MFC of the nanoemulsion form have been found to be lower than the non-nanoemulsion form ($P<0.05$). Among the studied groups, the nystatin group had been found to have the best effect ($P<0.05$).

Conclusion: Considering the different antifungal mechanisms and also the different resistance mechanisms of nystatin and *Hypericum perforatum* nanoemulsion, the prepared nanoemulsion can be used to treat and reduce the population of nystatin-resistant *Candida albicans* strain. However, the efficiency of these materials needs to be confirmed in larger studies on resistant strains.

Keywords: Nanoemulsion, *Hypericum perforatum*, Antifungal effects, *Candida albicans*, Acrylic resin bases, Oral stomatitis, Dentistry.

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1. INTRODUCTION

Acrylic resin is a thermoplastic or thermoset material that is usually obtained from acrylic acid, methacrylic acid, and acrylate monomers, such as butyl acrylate, or methacrylate monomers, such as methyl methacrylate [1]. However, one of the problems of acrylic resins despite the improvement of physical and chemical properties is their microbial infections [2].

Oral stomatitis is one of the common infections related to dentures (complete or partial), which is seen in the form of diffuse inflammation, mainly with *Candida albicans*. Its symptoms include inflammation and erythema of the oral mucosa covered by dentures [3, 4]. The incidence of denture stomatitis is higher among the elderly and women [3]. Etiological factors include improper prosthesis hygiene, continuous and nightly use of removable prostheses, accumulation of microbial plaque in artificial teeth, and bacterial and yeast contamination of the prosthesis surface. In addition, improperly fitting dentures can increase mucosal trauma and aggravate symptoms. Candida yeasts are the most common etiological agent of fungal infections of the oral cavity [5]. In addition, Candida-related prosthetic stomatitis, a mucosal inflammatory process under a removable denture, is the most common appearance of oral candidiasis among removable denture users [6].

The most common type of chronic oral candidiasis is *Candida albicans*, which is known as the most vital microorganism in this condition. All the mentioned factors seem to increase the ability of *Candida albicans* to colonize on denture and mucosal surfaces. Biofilm formation is an obvious and essential characteristic of the presence of *Candida albicans* in the oral cavity [7]. *Candida albicans* biofilms have various virulence factors that can increase their pathogenesis and are clinically problematic because some species are resistant to antifungal agents [8].

Antifungal treatment can eradicate *Candida albicans* infection and relieve the symptoms of stomatitis. However, if the artificial teeth are not disinfected and their cleanliness is not maintained, stomatitis may recur after stopping the antifungal treatment [9].

Recent advances in prosthetic materials focus on tools to reduce the development of adherent biofilms. The existing methods are effective in reducing the colonization of bacteria and yeast, and by observing the proper hygiene of the dentures, denture stomatitis can be reduced. Common treatment methods that have been suggested so far include the use of chemical drugs, such as nystatin, fluconazole, and amphotericin [10].

Due to the increase in antifungal resistance compared to antifungal drugs, other substances have been considered [11, 12]. *Hypericum perforatum* is a herbaceous plant belonging to the *Clusiaceae* family, which is used in traditional medicine and herbal therapy due to its antiseptic effects. It has shown antibacterial, antiviral, anti-inflammatory, and analgesic activities. *Hypericum perforatum* extract contains flavonoids and phenolic acids that show free radical scavenging activity [13, 14]. *Hypericum perforatum* extract has been shown to exert effective anti-inflammatory effects on animal models with acute inflammation [9].

Due to the creation of a resistant form of *Candida albicans*, the use of conventional drugs is not effective in some situations. Plants and natural bioactive compounds can be new sources of antifungal therapy. Some studies have shown herbal medicines and natural bioactive compounds to have antibacterial, antiviral, and antifungal effects [15-17]. Therefore, it is hypothesized that these natural products may have beneficial effects on pathogenic oral fungal flora, such as *Candida albicans*. Although the effects of medicinal plants as antifungal agents have been investigated in several studies, to the best of our knowledge, the effects of these natural products on *Candida* species have not yet been investigated. In general, herbs and nutrients, such as garlic, green tea, propolis, curcumin, licorice root, cinnamon, resveratrol, ginger, and berberine, have been found to be useful and can be considered in the treatment of *Candida albicans* in oral candidiasis. The aforementioned materials are used as an available and cheap option in an effort to prevent and treat oral diseases [18].

In recent years, the use of nanotechnology solutions has helped chemical and herbal medicines work better. Acrylics containing silver nanoparticles have shown good antifungal effects against *Candida albicans* [19, 20]. Various forms of nanomaterials are used for therapeutic applications. Nanoemulsions are biphasic dispersions of two immiscible liquids, water-in-oil or oil-in-water, stabilized by a biophilic (hydrophilic and lipophilic) surfactant. The use of nanoemulsions has increased significantly all over the world today, and their use in the treatment of oral stomatitis can also be investigated [19].

The aim of the present study was to investigate the antifungal effects of *Hypericum perforatum* nanoemulsion against *Candida albicans*.

2. METHODOLOGY

2.1. Sample Size

The study included four groups [*Hypericum perforatum* oil, *Hypericum perforatum* nanoemulsion, distilled water as a negative control (as a common negative antimicrobial material), and nystatin as a positive control (a gold standard drug against *Candida albicans*)].

Since we had prepared *Hypericum perforatum* in nanoemulsion form, the non-nano form of this material was placed among the groups to determine the effects of nanoization.

The number of tests was the average of three repetitions (12 total samples: three samples per group).

2.2. Preparation of Nanoemulsion

Hypericum perforatum nanoemulsion was prepared using ultrasonic emulsification. For this purpose, Tween 80 surfactant (2% w/w, Sigma-Aldrich, Louis, USA) was

slowly added to *Hypericum perforatum* oil (4% w/w, Barij essence, Tehran, Iran) and stirred in a mixer (1000 rpm/min, Heidolph, Schwabach, Germany) until complete dissolution. Then, the distilled water was slowly added to the mixture, and the mixture was stirred for 10 min until large emulsion droplets were formed. Then, the large droplet emulsion was transferred to the ultrasonic bath. It was subjected to an ultrasonic processor (Sonics VCX2500, Newtown, USA) at the power, frequency, and amplitude of 400 W, 20 kHz, and 40%, respectively, to convert the coarse droplets into nanometer dimensions [21].

2.3. The Antifungal Activity

Candida albicans standard strain was purchased from Pasteur Institute (Tehran, Iran). In order to determine the Minimum Inhibitory Concentration (MIC) of the prepared nanoemulsions, the broth microdilution technique was used employing Yeast Peptone Dextrose broth growth medium (YPD, Sigma-Aldrich, Louis, USA) inside the microplates. The prepared microplate was incubated for 24 hours at 37°C and then 50 cells from each well of the microplate were inoculated separately on dextrose agar growth medium. The first concentration of nanoemulsion at which no clear fungal growth was observed in the culture medium was considered the Minimum Inhibitory Concentration (MIC) and the first concentration in which the fungal growth was completely stopped and no fungal growth was observed on the culture medium plates was considered the Minimum Fungicide Concentration (MFC) [22].

2.4. Statistical Analysis

The results have been reported as descriptive statistics. The Shapiro-Wilk test was used to check the data normality. One-way ANOVA test was used to compare the antifungal effects between the groups. Tukey's post-hoc test was used to make comparisons between the groups. GraphPad software (Version 9, Boston, Massachusetts, USA) was used for data analysis. A probability value less than 0.05 was considered as a significant level.

3. RESULTS

It was observed that both the free form and nano form of the studied extract showed good antifungal activity against *Candida albicans*. The minimum inhibitory concentration and the minimum fungicide concentration of the nanoemulsion form were lower than the non-nanoemulsion form ($P<0.05$). Among the studied groups, the nystatin group had the best effect ($P<0.05$).

Tukey's post-hoc test showed the nanoemulsion to have a significant difference with free form ($P=0.001$) and nystatin exhibited a significant difference from both of them ($P=0.01$ and $P=0.0005$, respectively).

Table **1** shows the results of the comparison of the MIC and the MFC between the studied groups.

4. DISCUSSION

Over the past two decades, the incidence of fungal infections has increased dramatically, and the frequent failure of existing antifungal therapies represents a major clinical problem [10]. The urgent need for new antifungal drugs has drawn special attention to the study of natural products. In patients with complete dentures, problems, including the loss of compatibility of the denture with the jaw, trauma to the jaw while eating, lack of oral hygiene and dentures, the presence of opportunistic microorganisms, such as *Candida albicans* in the mouth, and stomatitis caused by dentures, are common. On the other hand, because the patients who use complete prostheses are mostly old, they may not have enough physical ability to fully observe hygiene due to other diseases, including Parkinson's [6], or because of Alzheimer's disease, they may not remember the health issues recommended to them by the doctor. Considering that the current topical treatments for candidiasis are nystatin, fluconazole, and in more severe cases, amphotericin B, issues, such as the side effects of using these drugs and the development of drug resistance, should also be considered for these types of topical treatments. Considering all the above issues, if it is possible to mix an antimicrobial substance in the acrylic during the preparation of the prosthesis, it can be said that the incidence of the mentioned problems would decrease [10].

Table 1. The comparison of MIC and the MFC between the studied groups.

Groups	MIC (µg/mL) (mean±SD)	One-way ANOVA (P-value)	MFC (µg/mL) (mean±SD)	One-way ANOVA (P-value)
Hypericum perforatum oil	106.66±30.16	0.0005	213.33±60.33	0.0001
Hypericum perforatum oil nanoemulsion [a, A]	42.66±15.08		85.33±30.16	
Negative control (distilled water)	0		0	
Positive control (nystatin)	13.33±4.71		33.33±9.42	

Note: a: significant differences with *Hypericum perforatum* oil.
A: significant differences with nystatin.

The microorganism used in this study was *Candida albicans* fungus. One of the main factors in causing stomatitis caused by dentures in patients is the opportunistic fungus *Candida albicans* [23].

The *Hypericum* plant includes many species that are used in traditional medicine to treat pathological conditions, such as inflammation and infection caused by fungi. However, despite the widespread use of *Hypericum*-based products, the antifungal potential of this species has not yet been well investigated. *Perforatum hypericum* extract is a rich source of flavonols, benzoates, cinnamates, and flavan-3-ols. Therefore, its antifungal properties can be attributed to the richness of *Perforatum hypericum* extract in these substances [24].

It was observed that both the non-nano form and nano form of this extract showed good antifungal activity

against *Candida albicans*. The nanoemulsion form provided better results than the non-nano form of the *Perforatum hypericum* oil. The minimum inhibitory concentration and the minimum fungicide concentration of the nanoemulsion form were lower than the non-nanoemulsion form ($P<0.05$). Among the studied groups, the nystatin group had the best effect ($P<0.05$).

Tuki and his colleagues evaluated five different species of *Perforatum hypericum* against a wide range of fungi isolated from patients with stomatitis. The studied species showed a broad antifungal activity against resistant fungi to *Penicillium*, *Aspergillus*, and *Candida albicans* [24]. In our study, both the non-nano form and nano form of this extract showed good antifungal activity against *Candida albicans*.

Najafi *et al*.'s study showed sodium bicarbonate to be very effective at low and medium concentrations of *Candida albicans* and showed a similar effect to nystatin and chlorhexidine, but at higher concentrations of *Candida albicans*, nystatin was found to be more effective [8]. In our study, the nystatin group exhibited the best effect among the studied groups.

Casemiro *et al*. showed that the addition of 5% and 2.5% of silver nanoparticles to acrylic resin exerted a high antimicrobial effect against *Candida albicans* [25]. Li and colleagues showed that nanosilver exhibited antifungal activity and prevented *Candida albicans* biofilm formation. In their study, the antifungal activity and the inhibitory effect on adhesion and biofilm formation by the prosthetic base resin containing nanosilver, especially at a higher concentration, were proven [2].

Kahramanlu *et al*. showed that in acrylic resins, increasing the concentration of silver nanoparticles led to a greater antimicrobial effect. Also, acrylic resins containing silver nanoparticles exhibited stronger anti-microbial properties on standard *Candida albicans* strain and generally less effect on hospital-isolated strains. In addition, it has been found that the more time passes after exposure of the bacterial suspension to acrylic resins containing silver nanoparticles, the less the residual antimicrobial effect is [7]. In our study, the nanoemulsion form had better results than the non-nano form.

It should be noted that the strain of *Candida albicans* used in this study was the standard strain. While in strains isolated from patients due to the possibility of previous exposure to the drug and the inducibility of resistance mechanisms, it is possible to observe antifungal effects at high concentrations (high MICs). Thus, due to the difference in inherent sensitivity, a significant difference in MICs of nystatin and the studied nanoemulsion was observed in this study.

Espinoza and his colleagues showed that the smaller the size of nanoparticles, the greater the antimicrobial and antifungal effects [26]. In our study, the nanoemulsion form demonstrated better results than the non-nano form of *Perforatum hypericum* oil. It should also be noted that *Candida albicans* is a Gram-positive species. The cell wall of Gram-positive species had between 3 and 20 layers of peptidoglycan more than Gram-negative species. Therefore, Gram-positive bacteria can be less sensitive to nanoparticles because their peptidoglycan layers are inactive [7].

It should also be considered that these types of nanomaterials can be used for the slow and long-term release of antifungal and antimicrobial drugs inside acrylic materials in dentistry. Soleymanijadidi *et al*. assessed the release pattern and the antimicrobial action of acrylic resins containing nanocurcumin. The release pattern showed a slow release pattern for all concentrations of nanocurcumin. The authors suggested that resins containing curcumin nanoparticles can be beneficial for antimicrobial purposes in dentistry applications [27].

Moreover, it could be interesting in the future to test *Hypericum perforatum* nanoemulsion in combination with other preventive treatments, such as ozone [28], photobiomodulation [29], and paraprobiotics [30] in order to understand their mutual effect on Candida and other fungal infections.

The limitation of this study is the use of *Hypericum perforatum* as an available and cheap option in an effort to prevent and treat oral diseases. Also, most of the evidence included has been based on *in vitro* and animal studies, so more clinical trials are needed.

CONCLUSION

The prepared nanoemulsion can be used to treat and reduce the nystatin-resistant *Candida albicans* strain. The long-term effects of nanoemulsion on acrylic, for example on its color change, can be investigated. It should be noted that the strain of *Candida albicans* used in this study was the standard strain. Therefore, in further studies, this nanoemulsion can be tested on nystatin-resistant species. It may also be possible to insert nanoemulsion into the acrylic structure and perform physicochemical and physicomechanical tests, as well as biocompatibility tests, and finally, the antifungal performance can be assessed in the laboratory, animal, and clinical stages.

AUTHORS' CONTRIBUTION

NY, MYM, and SMD contributed to the study conception and design; ME, AZ, and YP collected the data. All authors have reviewed the results and approved the final version of the manuscript.

LIST OF ABBREVIATIONS

MIC	=	Minimum Inhibitory Concentration
MFC	=	Minimum Fungicide Concentration
ANOVA	=	One-way Analysis of Variance

ETHICAL STATEMENT

The ethics committee for Tabriz University of Medical Sciences, Iran approved the ethics consideration (code: IR.TBZMED.VCR.REC.1402.132).

CONSENT FOR PUBLICATION

Not applicable.

AVAILAIBILITY OF DATA AND MATERIALS

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

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Demski S, Dydek K, Bartnicka K, *et al*. Introduction of SWCNTs as a method of improvement of electrical and mechanical properties of CFRPs based on thermoplastic acrylic resin. Polymers 2023; 15(3): 506. http://dx.doi.org/10.3390/polym15030506

[2] Li Z, Sun J, Lan J, Qi Q. Effect of a denture base acrylic resin containing silver nanoparticles on *Candida albicans* adhesion and biofilm formation. Gerodontology 2016; 33(2): 209-16. http://dx.doi.org/10.1111/ger.12142 PMID: 25070079

[3] Gendreau L, Loewy ZG. Epidemiology and etiology of denture stomatitis. J Prosthodont 2011; 20(4): 251-60. http://dx.doi.org/0.1111/j.1532-849X.2011.00698.x PMID: 21463383

[4] McReynolds DE, Moorthy A, Moneley JOC, Jabra-Rizk MA, Sultan HS. Denture stomatitis-An interdisciplinary clinical review. J Prosthodont 32(7)2023; : 560-70. http://dx.doi.org/10.1111/jopr.13687 PMID: 36988151

[5] Inácio Silveira DQ, Lia EN, Massignan C, Stefani CM. Natural products for the treatment of denture stomatitis: A systematic review. J Prosthet Dent 2023; 129(3): 413-24. http://dx.doi.org/10.1016/j.prosdent.2021.05.022 PMID: 34247854

[6] Preeti L, Magesh KT, Rajkumar K, Karthik R. Recurrent aphthous stomatitis. J Oral Maxillofac Pathol 2011; 15(3): 252-6. http://dx.doi.org/10.4103/0973-029X.86669 PMID: 22144824

[7] Ghahremanloo A, Movahedzadeh M. The effect of silver nano particles on candida albicans and streptococcus mutans in denture acrylic resins. J Dent Mater Tech 2016; 5(1): 23-30.

[8] Najafi S, *et al*. Effect of sodium bicarbonate against *Candida albicans* in denture stomatitis: An *in vitro* study. J Dent Med 2016; 29(2): 92-100.

[9] Saddiqe Z, Naeem I, Maimoona A. A review of the antibacterial activity of *Hypericum perforatum* L. J Ethnopharmacol 2010; 131(3): 511-21. http://dx.doi.org/10.1016/j.jep.2010.07.034 PMID: 20659547

[10] Motallebnejad M, Moghadamnia A. The efficacy of hypericum perforatum extract on recurrent aphthous ulcers. J Med Sci 2008; 8(1): 39-43. http://dx.doi.org/10.3923/jms.2008.39.43

[11] Gharibpour F, *et al*. The effects of nutraceuticals and herbal medicine on candida albicans in oral candidiasis: A comprehensive review. In: Barreto GE, Sahebkar A, Eds. Pharmacological Properties of Plant-Derived Natural Products and Implications for Human Health. Cham: Springer 2021; pp. 225-48. http://dx.doi.org/10.1007/978-3-030-64872-5_16

[12] Waterhouse A, Leslie DC, Lightbown K, *et al*. Rapid coating process generates omniphobic dentures in minutes to reduce *C. albicans* biofouling. ACS Biomater Sci Eng 2019; 5(2): 420-4. http://dx.doi.org/10.1021/acsbiomaterials.8b01214 PMID: 33405807

[13] Silva FA, *et al*. Phenolic acids and derivatives: Studies on the relationship among structure, radical scavenging activity, and physicochemical parameters. J Agric Food Chem 2000; 48(6): 2122-6. http://dx.doi.org/10.1021/jf9913110 PMID: 10888509

[14] Sroka Z, Cisowski WJF, Toxicology C. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem Toxicol 2003; 41(6): 753-8. http://dx.doi.org/10.1016/S0278-6915(02)00329-0 PMID: 12738180

[15] Stan D, Enciu AM, Mateescu AL, *et al*. Natural compounds with antimicrobial and antiviral effect and nanocarriers used for their transportation. Front Pharmacol 2021; 12: 723233. http://dx.doi.org/10.3389/fphar.2021.723233 PMID: 34552489

[16] Choudhary S, Zehra A, Mukarram M, *et al*. Potential uses of bioactive compounds of medicinal plants and their mode of action in several human diseases. In: Aftab T, Hakeem KR, Eds. Medicinal and Aromatic Plants. Cham: Springer 2021; pp. 143-58. http://dx.doi.org/10.1007/978-3-030-58975-2_5

[17] Sitarek P, Merecz-Sadowska A, Kowalczyk T, *et al*. Potential synergistic action of bioactive compounds from plant extracts against skin infecting microorganisms. Int J Mol Sci 2020; 21(14): 5105. http://dx.doi.org/10.3390/ijms21145105 PMID: 32707732

[18] Nasrollahi A, Pourshamsian K, Mansourkiaee P. Antifungal activity of silver nanoparticles on some of fungi. Int J Nanodimens 2010; 1: 3-239.: 233-9. http://dx.doi.org/10.7508/ijnd.2010.03.007

[19] Singh Y, Meher JG, Raval K, *et al*. Nanoemulsion: Concepts, development and applications in drug delivery. J Control Release 2017; 252: 28-49. http://dx.doi.org/10.1016/j.jconrel.2017.03.008 PMID: 28279798

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

[21] Wei S, Zhao X, Yu J, *et al*. Characterization of tea tree oil nanoemulsion and its acute and subchronic toxicity. Regul Toxicol Pharmacol 2021; 124: 104999. http://dx.doi.org/10.1016/j.yrtph.2021.104999 PMID: 34242706

[22] Rahmani Z, Karimi M, Saffari I, *et al*. Nanoemulsion and nanoencapsulation of a hydroethanolic extract of Nettle (*Urtica dioica*) and Wormwood (*Artemisia absinthium*): Comparison of antibacterial and anticancer activity. 2024; 12: 1266573. http://dx.doi.org/10.3389/fchem.2024.1266573 PMID: 38292020

[23] Tatapudi R, Abdul Samad SK, Manyam R, Dasari D, Lakshmi RV. Efficacy of curcumin in the treatment of denture stomatitis. J Oral Maxillofac Pathol 2021; 25(2): 286-91. http://dx.doi.org/10.4103/0973-029X.325128 PMID: 34703123

[24] Tocci N, Perenzoni D, Iamonico D, Fava F, Weil T, Mattivi F. Extracts from *Hypericum hircinum* subsp. *majus* exert antifungal activity against a panel of sensitive and drug-resistant clinical strains. Front Pharmacol 2018; 9: 382. http://dx.doi.org/10.3389/fphar.2018.00382 PMID: 29755350

[25] Casemiro LA, Martins CHG, Pires-de-Souza FCP, Panzeri H. Antimicrobial and mechanical properties of acrylic resins with incorporated silver–zinc zeolite – Part I. Gerodontology 2008; 25(3): 187-94. http://dx.doi.org/10.1111/j.1741-2358.2007.00198.x PMID: 18194331

[26] Espinosa Cristóbal L, *et al*. Antibacterial effect of silver nanoparticles against *Streptococcus mutans*.. Mater Lett 2009; 63: 2603-6. http://dx.doi.org/10.1016/j.matlet.2009.09.018

[27] Soleymanijadidi P, Moradi M, Hamedirad F, Ghanavati Z, Maleki Dizaj S, Salatin S. Nanocurcumin release from self-cured acrylic

resins; Effects on antimicrobial action and flexural strength. Bioengineering (Basel) 2023; 10(5): 559. http://dx.doi.org/10.3390/bioengineering10050559 PMID: 37237629

[28] Scribante A, Gallo S, Pascadopoli M, *et al*. Ozonized gels *vs* chlorhexidine in non-surgical periodontal treatment: A randomized clinical trial. Oral Dis 2023; 30(6): 3993-4000. http://dx.doi.org/10.1111/odi.14829 PMID: 38047757

[29] Elbay M, Elbay US, Kaya E, Kalkan OP. Effects of photobiomodulation with different application parameters on injection pain in children: A randomized clinical trial. J Clin Pediatr Dent 2023; 47(4): 54-62. http://dx.doi.org/10.22514/jocpd.2023.035 PMID: 37408347

[30] Butera A, Pascadopoli M, Nardi MG, *et al*. Clinical use of paraprobiotics for pregnant women with periodontitis: Randomized clinical trial. Dent J (Basel) 2024; 12(4): 116. http://dx.doi.org/10.3390/dj12040116 PMID: 38668028