



EFFECT OF PROBIOTICS AND PROPOLIS ON PLAQUE INDEX, PLAQUE PH, AND SALIVARY PH VALUES

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SUMMARY

Introduction: Despite preventive care, the prevalence of dental caries worldwide remains significant. This requires the search for and expansion of options for its prevention. Part of this effort is the addition of probiotic bacteria and propolis to various products for use in the oral cavity to reduce pathogenic microorganisms.

Purpose: The study aimed to evaluate and compare the effects of oral probiotic tablets and propolis mouthwash in children aged 7 to 12 on the pH values of dental biofilm and saliva and their potential for application as an element in the prevention of dental caries.

Results: The results show that 14 days of probiotic intake by children decreases plaque accumulation to values between 1.28 and 1.32. An increase in salivary and plaque pH values was also observed on the 7th and 14th day after regular use of probiotic tablets or propolis products. The table shows that both products participating in the study affected the plaque index and the pH levels in plaque and saliva in almost the same way, with no significant differences.

Conclusion: The present study results are in favor of the use of probiotics in children and suggest that oral administration of probiotics may help to increase plaque and salivary pH values with regular intake in children at average or high risk of developing caries, as well as to reduce plaque index among adolescents. Propolis mouthwash is a good alternative for children, especially those at high risk of developing caries. Propolis may be a promising agent for preventing oral diseases, demonstrated by the reduction of the plaque index and the increase of plaque and salivary pH.

Keywords: propolis, probiotics, oral health, preventive means,

INTRODUCTION

Despite preventive care, the prevalence of dental caries worldwide remains significant [1]. This requires the search for and expansion of options for its prevention. Part of this effort is the addition of probiotic bacteria and propolis to various products for use in the oral cavity to reduce pathogenic microorganisms.

Probiotics: The benefits of probiotics for human health have been discussed for decades [2]. Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [3]. Many studies confirm their important role in gastrointestinal health, and many authors are trying to prove their influence on the maintenance of oral health [2, 3]. The most commonly used probiotics include *Lactobacillus* and *Bifidobacterium*, which are part of the normal human microflora [2, 3].

Probiotics support oral health by maintaining microbial balance [4]. They can prevent the development of carious lesions by producing metabolites such as biosurfactants, bacteriocins, and extracellular polysaccharides, inhibiting adhesion and colonization and reducing the expression of virulence genes associated with biofilm formation by cariogenic pathogens [5]. Proper consumption of probiotics leads to a beneficial effect on the general and oral health of the individual [5]. They have the function of remaining on the surface of the teeth, competing with cariogenic microorganisms, and thus can inhibit the colonization and growth of those that cause caries [5]. In addition, they generate a biofilm that acts as a protective barrier against pathogenic bacteria and secrete various antimicrobial agents such as organic acids, hydrogen peroxide, and bacteriocins. This changes their surrounding environment by oxidizing and balancing the pH [5]. *Lactobacillus* probiotics effectively reduce the amount of *Streptococcus mutans* in saliva and increase salivary pH [6]. Together with postbiotics, they can play a stable and effective role in protecting the health of the digestive tract [7, 8].

Propolis: Herbal products such as *Acacia arabica*, eucalyptus extract, and propolis have been found to possess antibacterial agents and may be effective in the prevention and treatment of dental caries [9]. Propolis is a natural resinous material collected by honey bees, the composition of

which depends on the climate, season, location, and species of bees [10]. Propolis and its components have been extensively studied for their anti-inflammatory, antioxidant, antiulcer, antitumor, antidiabetic, cardioprotective, and local anesthetic effects [9-11]. Propolis is rich in bioactive components such as phenolic acids, flavonoids, amino acids, minerals, and vitamins. Its chemical composition includes 50% resin, 30% wax, 10% aromatic and essential oils, 5% pollen, and 5% other components [11]. These compounds have positive effects on the oral cavity and teeth. Since propolis possesses bacteriostatic, bactericidal, and anti-adhesion properties against a number of microorganisms, it is useful for reducing the amount of dental biofilm [12]. Flavonoids are the main biologically dynamic components of propolis extracts, which suggests the homogeneity of propolis preparations and makes their use harmless compared to many other synthetic products [13]. Ethanol extract of propolis has demonstrated antimicrobial activity against *Streptococcus mutans* and *Lactobacillus* spp., which have been assigned an essential role in the development of the carious process [12, 14]. The antimicrobial action of ethanol extract of propolis is based on the disorganization of the cytoplasmic membrane and cell wall, partial bacteriolysis, formation of pseudomulticellular colonies of bacteria, and inhibition of protein synthesis [12]. Using toothpaste or mouthwash with propolis ethanol extract can potentially reduce the formation of dental biofilm [15]. This means that propolis exhibits strong antimicrobial and anti-inflammatory properties, making it a good choice for treating and preventing oral diseases. After reviewing the available scientific literature, we found no studies investigating the impact of rinsing with propolis mouthwashes and oral probiotics in children aged 7-12 years on the pH values of dental biofilm, saliva, and plaque index.

PURPOSE

The **aim** of the study was to evaluate and compare the effects of oral probiotic tablets and propolis mouthwash in children aged 7 to 12 years on the pH values of the dental biofilm and saliva and their potential for application as an element in the prevention of dental caries.

MATERIAL AND METHODS

The study encompassed 40 children aged between 7 and 12 years who were at moderate or high risk of dental caries. The participants had no general diseases, allergies to milk, dairy, or bee products. They had not received antibiotic or corticosteroid treatment for at least one month before the commencement of the study. Informed consent was obtained from the parents of all participating children. The study received approval from the Ethics Committee of the Medical University of Sofia (No. 2296/28.06.2023).

The research was conducted in three stages with the same group of children, evaluating the effects of different products as follows:

- **Stage 1:** Control - children adhered to standard oral hygiene and dietary measures without additional instructions or prophylactic products;
- **Stage 2:** Administration of probiotic tablets once daily for 14 days;
- **Stage 3:** Rinsing with a propolis mouthwash once daily for 14 days.

During each stage, the child made three visits to the dental office - on the 1st day, on the 7th day, and on the 14th day. There was a one-month break between the individual stages before the start of the next. The products whose effect was assessed are described in Table 1. Each indicated product is approved and has an official permit for children's use and sale in drugstores, pharmacies, and grocery stores. Lactoflor BioPlus probiotic tablets are a food supplement officially registered under No. 23020168 in the official database of food supplements in Bulgaria. The manufacturer of the tablets is "Kendy Pharma", with headquarters in Bankya, 101 Sofia Street. The probiotic tablet was officially approved and registered for sale in the pharmacy network on 29.03.2021. It is recommended for intake by children over 3 years of age and is not a medicine but a food supplement that favorably improves the intestinal microflora and the immune system. According to the latest legislative provisions on cosmetic products in Bulgaria (Article 13 of Regulation (EC) No. 1223/2009), propolis mouthwash (Bio Apteka) was placed on the market as a cosmetic product after its notification in the European Commission's Cosmetic Products Notification Portal (CPNP).

Table 1. Composition of the studied products

Brand	Product	Composition
Lactoflor	Lactoflor BioPlus	Filler (isomalt), probiotic blend (1x10 ⁹ CFU/tab - Natural Bulgarian <i>Lactobacillus bulgaricus</i> LKZ-200, isolated from blood geranium leaves; Bulgarian <i>Lactobacillus rhamnosus</i> LLR-L1 with high bioavailability and increased activity, against candida/ e.coli/ st. Aureus; <i>Streptococcus thermophiles</i> , prebiotic (fiber-inulin), stabilizer (gum arabic), anti-caking agents (magnesium salts of fatty acids, silicon dioxide)
Bioapteka	Bio Apteka Mouthwash with honey and propolis	Aqua, Propolis Extract, Glycerin, PEG-40 Hydrogenated Castor Oil, Organic Honey Extract, Aroma, Zinc Gluconate, Sodium Saccharin, Pentylene Glycol, Methyl Diisopropyl Propionamide, Citric Acid, Potassium Sorbate, Sodium Benzoate

Oral examination: Each child underwent an oral examination by a pediatric dentist according to a standard protocol. Dental status was recorded, the DMFT index was calculated, and caries risk was determined. For all children studied, the Silness and Loe plaque index, saliva pH, and plaque pH were calculated using the methods described below. At the end of the first visit, professional oral hygiene was performed, followed by the patients' instruction and training on oral hygiene at home. All children were given identical toothbrush and toothpaste without fluoride. Recommendations were also made for maintaining a proper diet and oral hygiene regimen during the study.

Conducting the study: The study had three stages (Table 2). Stage 1 served as the baseline control. There was a 1-month interval between stages, during which children used no additional prophylactic products and maintained their usual nutrition and oral hygiene to avoid residual effects. In stages 2 and 3, each child used the respective product daily for 14 days as instructed. Control examinations took place in the morning from 8-11 am to avoid circadian rhythm effects. Children were instructed to avoid food and drinks (except water) for at least one hour before the study and not brush their teeth that morning. Parents supervised compliance with these recommendations.

Table 2. Distribution of children by stages of the study and regimen of intake of the studied products.

Stage	Number of children	Product	Instructions for usage
1	40	Control	Without taking a particular product
2	40	Probiotic lozenge	The lozenges are used once a day—in the morning, after regular oral hygiene. They are chewed and then swallowed.
3	40	Propolis mouthwash	Propolis mouthwash is used once a day. Twenty ml of the propolis mouthwash is gargled for 30 seconds after brushing your teeth.

Methodology for recording and assessing the plaque index: The Silness and Loe plaque index was used to determine the oral hygiene status of the child. The vestibular surfaces of all fully erupted maxillary incisors and canines (primary or permanent) were examined. Each representative tooth was inspected for plaque visually and scraped with a probe on the cervical third of its vestibular surface.

Methodology for saliva pH testing: A product by GC, Saliva-Check Buffer, was used. The test follows the manufacturer's instructions. Children were rinsed with water, then unstimulated saliva was collected in a sterile measuring cup (GC Saliva-Check Buffer). To measure the pH value of the collected saliva, litmus paper (GC Saliva-Check Buffer), included in the kit, is used.

Methodology for assessing the pH of dental biofilm: The pH level of children's supragingival dental plaque was monitored. Plaque was collected from the gingival third of the molars' vestibular surface using a sterile instrument. The amount of plaque was the size of the head of a pin-

head (1 mg). It was dissolved in 2 ml of distilled water in a sterile Eppendorf tube and mixed with a Biosan V-1 plus vortex mixer at 3000 RPM for 30 seconds. The pH was measured using an Oakon pHTestr 50S Spear-Tip Waterproof Pocket tester. The plaque index, the pH value of the saliva and the pH value of the dental biofilm were tested as follows:

- First stage (control) - on the 1st, 7th, and 14th days;
- Second stage - on the 1st, 7th, and 14th days;
- Third stage - on the 1st, 7th, and 14th days.

Statistical analysis was conducted using SPSS v.19.0 (SPSS Inc., Chicago, IL, USA). The significance level was set at $p=0.05$. Nonparametric tests were used to analyze and compare the results.

RESULTS

Table 3 shows the impact of probiotic tablet use among children aged 7 to 12 years for 14 days of oral health and compares the data with the baseline results.

Table 3. Effect of probiotic tablets on plaque index, salivary pH, and plaque pH value

Factor \ Time	1 day Mean ± SD	7 day Mean ± SD	14 day Mean ± SD
Plaque index (Silness & Loe)			
Baseline	2.19 ± 0.75	2.06 ± 0.72	2.12 ± 0.77
Probiotic tablet	2.05 ± 0.45	1.64 ± 0.55	1.32 ± 0.56
Paired Samples test	T=1.10, p=0.27	T=2.88, p<0.001	T=5.81, p<0.001
pH of saliva			
Baseline	6.87 ± 0.25	6.81 ± 0.50	6.80 ± 0.31
Probiotic tablet	6.93 ± 0.20	7.15 ± 0.52	7.22 ± 0.56

Paired Samples test	T=-2.08, p=0.06	T=-3.62, p<0.001	T=-4.61, p<0.001
pH of plaque			
Baseline	6.91 ± 0.36	6.92 ± 0.42	6.88 ± 0.41
Probiotic tablet	6.87 ± 0.33	7.04 ± 0.60	7.07 ± 0.60
Paired Samples test	T=0.20, p=0.84	T=-1.11, p=0.27	T=-1.61, p=0.11

The results show that 14 days of probiotic intake by children decreases plaque accumulation to values of about 1.32. The data are supported by statistically significant differences compared to baseline data (p<0.001). An increase in salivary and plaque pH values was also observed on the 7th and 14th days after regular use of

probiotic tablets.

Table 4 shows the impact of the use of propolis mouthrinse on oral health among children from 7 to 12 years for a period of 14 days and compares the data with the baseline.

Table 4. Effect of propolis mouth rinse on plaque index, salivary pH, and plaque pH values

Factor \ Time	1 day Mean ± SD	7 day Mean ± SD	14 day Mean ± SD
Plaque index (Silness & Loe)			
Baseline	2.19 ± 0.75	2.06 ± 0.72	2.12 ± 0.77
Propolis mouth rinse	1.74 ± 0.51	1.41 ± 0.58	1.28 ± 0.48
Paired Samples test	T=3.11, p<0.001	T=3.83, p<0.001	T=5.52, p<0.001
pH of saliva			
Baseline	6.87 ± 0.25	6.81 ± 0.50	6.80 ± 0.31
Propolis mouth rinse	6.92 ± 0.34	7.13 ± 0.57	7.19 ± 0.60
Paired Samples test	T=-0.68, p=0.49	T=-2.51, p=0.01	T=-3.50, p<0.001
pH of plaque			
Baseline	6.91 ± 0.36	6.92 ± 0.42	6.88 ± 0.41
Propolis mouth rinse	6.89 ± 0.45	6.99 ± 0.51	7.20 ± 0.63
Paired Samples test	T=0.20, p=0.84	T=-0.82, p=0.41	T=-2.63, p=0.01

Table 4 shows that propolis mouth rinse leads to a decrease in plaque index values and an increase in salivary and plaque pH after 2 weeks of regular usage.

Table 5 compares the effect of propolis and probiotic lozenges on oral health.

Table 5. Comparison of the effects of propolis and probiotics

Factor \ Time	1 day Mean ± SD	7 day Mean ± SD	14 day Mean ± SD
Plaque index (Silness & Loe)			
Probiotic tablet	2.05 ± 0.45	1.64 ± 0.55	1.32 ± 0.56
Propolis mouth rinse	1.74 ± 0.51	1.41 ± 0.58	1.28 ± 0.48
Paired Samples test	T=-2.683, p<0.001	T=-1.772, p=0.07	T=-0.043, p=0.96
pH of saliva			
Probiotic tablet	6.93 ± 0.20	7.15 ± 0.52	7.22 ± 0.56
Propolis mouth rinse	6.92 ± 0.34	7.13 ± 0.57	7.19 ± 0.60
Paired Samples test	T=-0.099, p=0.92	T=-0.056, p=0.95	T=-0.324, p=0.74
pH of plaque			
Probiotic tablet	6.87 ± 0.33	7.04 ± 0.60	7.07 ± 0.60
Propolis mouth rinse	6.89 ± 0.45	6.99 ± 0.51	7.20 ± 0.63
Paired Samples test	T=-.055, p=0.956	T=-0.23, p=0.812	T=-0.45, p=0.64

The table shows that both products participating in the study affected the plaque index and the pH levels in plaque and saliva in almost the same way, with no significant differences ($p>0.05$).

DISCUSSION

One of the emerging approaches currently being investigated for caries prevention is probiotics. According to some authors, probiotics can compete, antagonize, and prevent the spread of cariogenic bacteria, thus helping to prevent dental caries [3]. Oral probiotics are commercially available in tablets, lozenges, lyophilized powder, candies, ice creams, etc., and some authors use various dairy products with probiotics in their studies (curd, yogurt, cheese). Our data showed that the intake of probiotic tablets by children aged 7 to 12 years leads to an increase in plaque and salivary pH values compared to baseline data (Table 3), which would positively impact oral health. Regular probiotic intake can also potentially reduce the Silness and Loe plaque index (Table 3). Oral health is an integral part of general health and can be achieved through good oral hygiene [5, 6]. Maintaining oral hygiene is directly related to controlling plaque in the oral cavity. There are various methods for plaque control, among which mechanical control plays a significant role, and chemical control may have an additional role [5, 6]. Chemical control of plaque includes antibiotics, enzymes, bisguanides, metal salts, etc., and the products we studied can be attributed to them.

Probiotics can influence oral ecology by specifically preventing other bacteria's attachment and modifying the salivary pellicle's protein composition [6]. A study involving probiotic yogurt with probiotic strains *Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* showed that it is suitable for consumption due to its ability to buffer the environment. After 15 days of yogurt consumption, the study found a significant decrease in *Streptococcus mutans* in the study group [16]. The number of *Streptococcus mutans* decreased, and the saliva pH increased after taking probiotic tablets [17]. These data are also consistent with the results obtained in our study (Table 3). Other studies have also reported decreased plaque index after regular intake of probiotic tablets and other probiotic products [18].

Probiotics appear to have a similar effect to chlorhexidine and may, therefore, be recommended for use in young children at high risk of caries, in whom chlorhexidine solutions are not recommended [19]. The effect of probiotic tablets on plaque may be due to their ability to prevent adhesion and growth of microorganisms on the tooth surface and to reduce cytotoxic products by modifying the biochemical composition of plaque [3]. In the present study, mean salivary and plaque pH values increased at day 14 (Table 3). These results align with those of other authors who reported a significant increase in salivary pH values after consuming probiotic curd [20]. The in-

crease in saliva pH values after taking probiotic tablets can be explained by the arginolytic nature of some strains of lactobacilli (contained in probiotics), which have the potential to hydrolyze arginine found in saliva and convert it into ammonia, which maintains the pH value above 7 [10, 11].

Pharmaceutical companies have long been trying to promote natural oral care products [10]. Among them is propolis, a natural resinous material honey bees produce as an alternative anti-plaque mouthwash. Propolis has shown strong antimicrobial and anti-inflammatory properties, making it a good choice for treating and preventing oral diseases [10]. Several studies have investigated propolis's efficacy on plaque and reported mixed results. According to some authors, propolis shows an effect on the plaque index similar to that of chlorhexidine [20]. Propolis has general medical and oral positive effects and is used mainly for its antibacterial, antibiotic, anti-inflammatory, antifungal, antiviral, and antioxidant properties [21]. One study recorded a significant reduction in the plaque index - by about 44.7% after treatment with a mouthwash containing propolis, compared to a placebo mouthwash [22].

Furthermore, the authors found that propolis mouthwash could reduce the concentration of insoluble polysaccharides in plaque by approximately 61.7% [22]. The group treated with placebo mouthwash showed less than 5% plaque-free surfaces, while the experimental group recorded approximately 30% [22]. One of the mechanisms by which propolis mouthwash may affect plaque formation may be related to the inhibitory effect of propolis SNB-RS on microbial cell glucosyltransferase [22]. This explains the plaque reduction demonstrated in our and several other studies [22]. Glucosyltransferases are important agents that affect plaque accumulation. The water-insoluble glucan facilitates the adsorption of bacteria on teeth and creates a stable bond between streptococci and the dental pellicle. Thus, it increases the accumulation of dental plaque and the adhesion of bacteria to tooth surfaces. Apigenin, a 4',5,7-trihydroxyflavone, is an important component of propolis, responsible for inhibiting the glucosyltransferase activity of *Streptococcus mutans* [23]. Our study evaluated the effect of propolis mouthwash on plaque and salivary pH levels and recorded an increase in the values (Table 4). The authors studied the effect of different chewing gums, one of which contained propolis [23]. They found that plaque pH increased from a value of 6.98 at the beginning of the study to 8.35 one month after the start of the experiment [23]. The authors found no differences in salivary pH levels at the beginning and the end, and the pH level remained above 7.4 - within the neutral range [23]. The use of propolis in dentistry has a good potential therapeutic effect [24]. Water supplemented with ethanolic extract of propolis as a mouthwash has been shown to reduce dental plaque [24]. The beneficial effect of reducing dental plaque retention has been found in using propolis toothpaste [25].

CONCLUSION

The present study results are in favor of the use of probiotics in children and suggest that oral administration of probiotics may help to increase plaque and salivary pH values with regular intake in children at moderate or high caries risk, as well as to reduce plaque index among adolescents.

Propolis is a natural and reliable antimicrobial alternative to chlorhexidine, which, despite its antibacterial properties, has contraindications and cannot be used

for a long time due to the typical staining of the teeth and tongue. Propolis mouthwash is a good alternative for children, especially those at high risk of developing caries. Propolis may be a promising agent for preventing oral diseases, demonstrated by the reduction of the plaque index and the increase of plaque and salivary pH.

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