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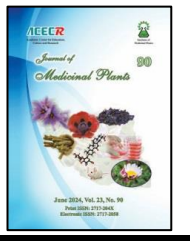

Research Article

Anti-caries efficacy of the medicinal plant Miswak (*Salvadora persica* L.) and propolis Varnish on extracted human Premolar and Molar teeth

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ABSTRACT

Background: Tooth decay is the result of the activity of cariogenic microorganisms that live in dental plaque. Among these, *Streptococcus mutans* is the main factor in the development and progression of dental tissue destruction. **Objective:** The study aims to investigate the anti-caries potential of a natural compound-based varnish against *S. mutans* on a set of dental specimens. **Methods:** Fifty-four extracted human molar and premolar specimens were procured from an outpatient clinic of pediatric dentistry, Bojnurd, Iran. These teeth were cast in a silicon putty and treated with a varnish made of propolis and miswak (from twigs of *Salvadora persica* L. tree) ethanolic extracts, followed by incubation in a harsh acidic medium (pH 2). Then, the density of samples, antibacterial activity, buffering capacity, and fluoride release were respectively investigated using radiography, disc diffusion and microdilution tests, and pH drop challenge in acetic acid solution (PH 4). **Results:** Overall, the varnishes protected the dental samples from dissolution in the acidic medium, as evidenced by the radiographic examination. The varnishes also exhibited remarkable antibacterial activity (inhibition zones of 3.2 ± 0.8 mm and 2.0 ± 0.2 mm for miswak and propolis varnish films), a considerable buffering capacity (pH increase from 4 to 7 within 5 minutes), and released fluoride (mean 85 µmoles). **Conclusion:** The application of natural compound-based varnish is strongly recommended for dental protection.

1. Introduction

Tooth caries are the dire consequence of the cariogenic microorganisms that reside in the dental plague [1]. Of note, *Streptococcus mutans* is the characteristic culprit in the establishment and progression of dental tissue

Abbreviations: MEE, miswak ethanolic extract; PEE, propolis ethanolic extract

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destruction [1]. Along with other pathogenic microbiota in the dental plaque, *S. mutans* encourages the underlying osseous tissue demineralization via its acidic metabolites (mainly lactate) under anaerobic conditions [2]. These metabolites are produced following a glucose challenge, and their accumulation advances the gradual disintegration of the dental tissue [2].

To restrict the mentioned cariogenic process, the biotic and abiotic factors need to be addressed. To this end, one of the conventional dental-care practices is to remove the dental plagues through conventional bleaching techniques [3] and then cover the enamel surfaces with protective varnishes [4]. These varnishes contain a resinous ingredient that provides a hard coating on the dental surface [5-7]. The coating physically separates the dental surface from the mouth environment and prevents the re-establishment of fresh dental plague. In the meantime, the separation allows for the gradual restoration (remineralization) of the tooth's mineral tissues [8].

In addition, varnishes usually contain high doses of fluoride sodium, which result in the formation of acid-resistant mineral tissue during the remineralization process [9]. The presence of fluoride ions allows for the formation of fluorapatite and the replacement of acid-sensitive hydroxyapatite in the restored tissue [9].

Additional anti-cariogenic agents that restrict the growth and activity of *S. mutans* are also used for oral hygiene [7, 10, 11]. A wide assortment of antibacterial and antimicrobial agents has been utilized in various forms in dental health products [12]. In recent years, the use of natural compounds has been encouraged as a substitute for chemical antibacterial agents in several pharmaceutical products, including varnishes [4, 7, 11]. Propolis, a beehive product, is a resinous substance with high activity against *S. mutans* and adhesiveness to tooth surfaces [13]. Miswak is a teeth-cleaning root or twig from the Arak (*Salvadora persica*) tree. Miswak is traditionally used for cleaning teeth in many cultures, especially in Muslim-majority regions. It possesses high amounts of tannic acids and alkaloids known for their wound-healing and antimicrobial potential [7]. Both of these agents are outstanding candidates for use in varnishes.

The frequent anaerobic activity of *S. mutans* is accompanied by acidity in dental plague [2]. Evidence shows that the acidity of the plague reduces to pH 4 after a glucose challenge [2]. The drop in pH is attributed to the production of acidic metabolites (e.g. lactic acid and propionic acid) under anaerobic conditions and is enough to promote demineralization [2].

Considering the potential role of the ingredients, the current study aims to investigate the anti-cariogenic properties of a Propolis/Miswak-based varnish.

2. Materials and methods

2.1. Materials

All bacterial culture and chemical reagent were obtained from Merck, Germany, including triphenyl Tetrazolium Choride reagent and sodium fluoride. *S. mutans* ATCC 25175 was obtained from Microbiologics, St. Cloud, Minnesota, USA.

2.2. Preparation of extracts

Miswak and propolis were extracted with ethyl alcohol (95 %) and dried at room temperature [7]. Miswak twigs were collected from the shores of the Persian Gulf and procured by an herbal shop in Mashhad, Iran. Their authenticity was independently verified by the herbarium at Mashhad University of

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Medical Sciences in Mashhad, Iran, where the voucher specimen was deposited under herbarium number 12540. They were ground into a fine powder, of which 10 g were immersed in 95 % ethanol (25 ml) at room temperature. The supernatant was taken every day and replaced with fresh solvent. On the fourth day, the collected supernatant was then passed through a 0.45 mm filter paper, and the solvent was completely removed in a vacuum-equipped rotary evaporator at 45°C, followed by overnight freeze-drying. The resulting dried miswak ethanolic extract (MEE) was kept at 4 °C until use.

For the propolis ethanolic extract (PEE), 50 g of commercial propolis fine chips, collected from the hives of honey bees (*Apis mellifera carnica* L.), were dissolved in 500 ml of 95 % ethyl alcohol and left for 4 days in the dark with occasional stirring [7, 14]. The mixture was filtered through 0.45 mm filter paper, and the filtered solution was placed in the vacuum-equipped rotary evaporator to remove the solvent. The remainder was transferred into a set of Petri dishes and left at room temperature until the product achieved a honey-like consistency. The PEE was then stored at 4 °C.

2.3. Preparation of varnishes

The natural compound-based varnishes were prepared in ethyl alcohol/tris-buffered saline/sodium fluoride (75/25 % v/v; TBS, 50 mM, pH ~ 7.4; NaF, 5 % w/v) at equal concentrations (5 g/l) of MEE, PEE, and colophony according to table 1. For this purpose, an appropriate volume of ethyl alcohol/TBS/NaF was added to MEE, PEE, and the colophony powder, agitated, and probe-sonicated for 15 min at 60 °C. The final varnishes were kept in the dark at room temperature in sealed/capped vials until use.

Table 1. varnish preparations and anti-cariogenic properties

Varnishes nomenclature	Formulations	F release (µg/ml)	Inhibition zone (mm)	pH post-acid challenge
MEE/NaF V.	MEE/Colophony/NaF/TBS	92 ± 23	3.2 ± 0.8	7.0 ± 0.0
PEE/NaF V.	PEE/Colophony/NaF/TBS	86 ± 18	2 ± 0.2	7.3 ± 0.1
NaF V.	Colophony/NaF/TBS	86 ± 15	0	7.1 ± 0.1
Non-buffered V.	PEE/MEE/NaF	77 ± 12	2.4 ± 1.1	4.4 ± 0.0

2.4. Antibacterial susceptibility test

The antibacterial activity of the varnish ingredients was assessed on *S. mutans* ATCC 25175 using the disc diffusion and broth microdilution susceptibility methods [15-17].

For the disc diffusion, 30 ml of freshly prepared and autoclaved TSA (40.0 g/l) was distributed into separate Petri dishes under sterile conditions and left to cool to room temperature. Subsequently, a sample of *S. mutans* was taken from the bacterial suspension with turbidity equal to 0.5 McFarland standards by a sterile loop and streaked across the agar. Next, 6.4 mm blank paper discs (Padtan Teb Co., Iran) were covered with the varnish formulations and ingredients and left for half an hour under sterile conditions to dry. The discs were then turned and placed on the Petri dishes. These Petri dishes were incubated for 48 h for bacterial colonization at 35 °C. Finally, the halo inhibition zones (2r, mm) were recorded for the discs using a ruler.

The microdilution experiment was conducted with the *S. mutans* suspension prepared in TSB. One hundred microliters of the bacterial suspension were transferred to 96-well U-bottom plates. Next, the double dilutions (0.1 ml) of the varnishes and extracts (in DMSO)

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were added in quadruplicate. After incubating for 48 h at 35 ºC, triphenyl tetrazolium chloride reagent (0.050 ml, 10 % w/v in distilled autoclaved water) was added to each well. The plate was incubated again for a further 24 h in the incubator, and then the purple color development in the wells was measured with an ELISA reader (BOECO, Germany) at 625 nm to obtain the minimum inhibitory concentration (MIC) of the reagents. For the minimum bactericidal concentrations (MBC), samples were taken from the wells without bacterial activity and incubated on TSA for 48 h to test their colonization potential.

2.5. Specimens

The dental samples were prepared and processed from clinically caries-free human premolar teeth (obtained from the outpatient clinic of the Pediatric Dentistry Department, Faculty of Dentistry, North Khorasan University of Medical Sciences, Bojnurd, Iran). First, the extracted teeth were cleaned and bleached for the removal of the plague. The teeth were then examined closely under a stereomicroscope for the exclusion of the teeth with cracks or cavitation and those with unsound enamel surfaces. Subsequently, the roots were removed and the teeth were dissected into buccal and lingual halves using a dental lab die-cutting machine (Threedental^{TM}, China). The prepared specimens were autoclaved and stored in normal saline until use. All the surfaces were covered twice with acid-resistant nail varnish, except for the enamel surface that was covered with the test varnishes. Thereafter, the enamel specimens were randomly distributed into the experimental groups (9 specimens each), and they were cast in silicone putty (activated with the universal activator, Speedex activator, Coltene groups, Switzerland), where the putty film was the size of the standard periapical film (size 2, 3×31×41 mm^3) and the enamel surfaces were exposed on top.

2.6. Radiographic examinations

The varnishes were applied to the dental experimental units, whose demineralization was examined in an accelerated experimental caries-promoting environment (KCl buffer; 0.1 M, pH~2). To this end, the mineral content of the teeth was analyzed radiographically before and after treatment with the acidic media [18]. For this purpose, the enamel surface of the dental samples was coated twice with the varnishes with a paintbrush (flat size, 3) with a 30-minute resting period in distilled water in between. The samples were irradiated on the day of applying varnishes and at the end of the acid treatment week (Fig. 1). The digitized RVA radiographs were collected. All the shots were taken at a fixed x, y, and z coordinate from the irritating tube (Fig. 1 Aa) with the help of a hand-made holder in a box (Fig. 1 Ab), which positioned the putty platform and the radiologic sensor in a fixed coordinate relative to each other. Also, for all the samples, the irradiation was done under the same conditions (exposure time, 0.12 s; Heliodent 70®, 70 kV, 7 mA, Siemens, Germany).

The obtained radiographs were subsequently digitized using the computer-assisted imaging system (Fig. 1 B). The computed digital RVG images were recorded in grayscale mode (with a range of 0 to 255, 8-bit). For the radiographic calibration of the images, the radiographic grayscale data of a plastic button and the silicon putty platform (Fig. 1 Bc) were employed as the reference points in the regression analysis of the data. The entire area of the pre-test dental specimen was selected for the grayscale quantitative analysis (Fig. 1 Bd), which was geometrically juxtaposed with the same area surrounding the post-test sample (Fig. 1 Be). The grayscale calculated mean of the areas of

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the pre-test (Fig. 1 Bf) and pot-test samples (Fig. 1 Bg) was taken as the data, and this calculation procedure was conducted for every one of the samples' radiographs.

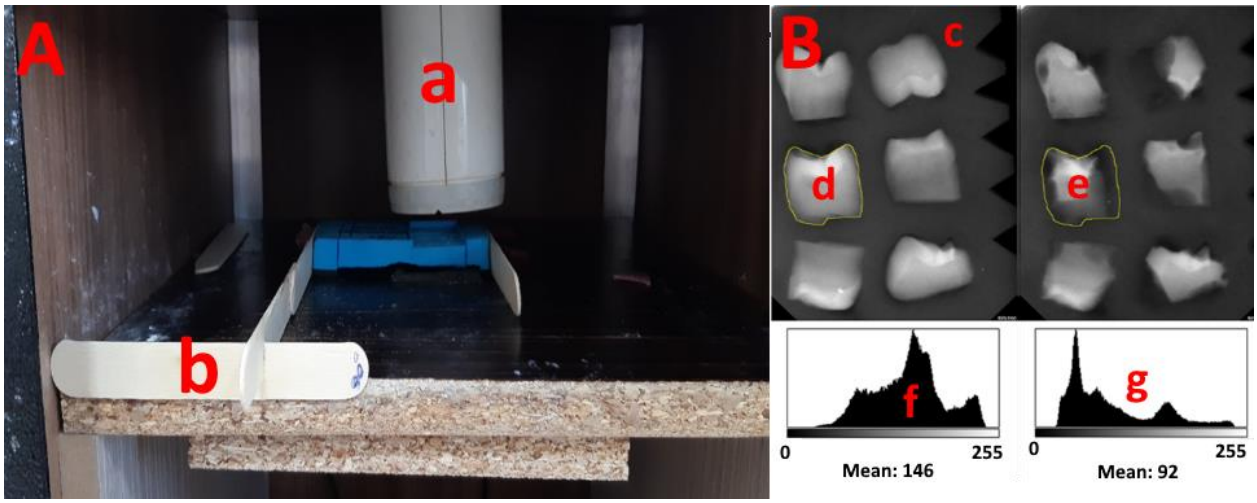

Fig. 1. The irradiating tube at a fixed angle (A) and radiographs of the dental samples (B). The irradiating tube (a) was positioned at a fixed coordinate using the handmade holder (b) in a wooden box. The radiographs of the dental samples (already cast in silicon putty, c) were taken before and after the incubation period in the acidic condition. Using computer-assisted software, the area related to the dental samples was selected individually in the radiograph of the pre-incubated samples (d), and superimposed on the post-incubated samples (e). Subsequently, the geometric means of the grayscale histograms of the pre-incubated (f) and post-incubated samples (g) were taken as the quantitative data.

2.7. Buffering capacity of varnish films

Varnish buffering capacity was assessed with the varnish films against extremely diluted acetic acid (0.01 mM; pH, 4.2; T, 25 ^{o}C). Flat-bottom 12-well culture plates were covered using 0.1 ml of vanishes and the plates were left for the film to toughen up under laminar air circulation. This step was repeated one more time, and then 0.1 ml of normal saline was added to the well to cover the film. Again, the plate was left to dry. Next, 0.3 ml of the acetic acid solution was added, and the pH was measured for 5 minutes with a pH meter (InoLab pH7110, Germany). For control, non-buffered MEE/PEE varnish film was used.

2.8. Fluoride leakage from varnish films

The varnish film was also assessed in terms of fluoride leakage from the film in the acetic acid solution using the ion meter [19]. As with the previous section, "buffering capacity of varnish films," a 12-well plate was covered with the varnish films, and the concentration of the released fluoride ion was measured in the media according to the fluoride standard solutions (NaF in deionized water, 0.02-1.0 mg/l). For this purpose, 1 ml of the acetic solution was added to the wells, and the plate was incubated for one week at 37^{o}C. Subsequently, the samples were mixed with 9 ml of an ionic strength adjustment buffer (CDTA, 4 g/l, glacial acetic acid, 57 ml; NaCl, 58 g/l; all in deionized water, pH~5), and their fluoride ion concentration was recorded with the Metrohm Ion meter against the fluoride standards in direct mode.

2.9. Sample size

The experiments were conducted with a study power of 80 % and a statistical significance of 5 % (α = 0.05). Three samples were estimated

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for each varnish group, where varnishes were tested without the use of dental experimental units. The mean ± SD of the inhibition zone (mm) for PEE and chlorhexidine were 20.5 ± 0.33, and 18.5 ± 0.55, respectively [20], and the mean ± SD of fluoride release was 34.9 ± 0.3 from varnish at week one [19].

For the radiographic examination of the teeth, 9 enamel specimens per group were estimated based on the mean ± standard deviation of grayscale values for the radiographs of the controlled non-treated (50 ± 13) and Preventa varnish group (129 ± 12).

2.10. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 8 (GraphPad Software, San Diego, CA) at a significance level of 5 %. One-way ANOVA was carried out among the varnish groups for their antibacterial effect, buffering capacity, and fluoride leakage. Once the ANOVA test became significant, Newman–Keuls multiple comparisons test was employed to find out the significant differences between the groups.

The radiograph grayscale data were analyzed using two-way statistical analysis. When the difference between groups was significant, Newman–Keuls multiple comparisons test was employed to find out significant differences between pre- and post-incubated samples, and also between the samples at the end of the incubation period.

3. Results

3.1. The yield of extraction and solubility

The extraction from miswak yielded double the amount of extract compared to propolis (4.66 ± 0.41 % and 2.13 ± 0.15 % w/w respectively). Similarly, MEE had higher solubility in ethyl alcohol/water solvent compared to PEE (160.0 g/l versus 5.3 g/l).

3.2. Antibacterial susceptibility

Table 2 shows the antibacterial activity of MEE and PEE in broth and agar media. A large inhibition zone developed around the disc (12.1 ± 1.2 mm) of MEE (lower than that of chlorhexidine 17.0 mm), while PEE and other reagents showed no inhibition zone around their discs.

On the other hand, PEE exhibited antibacterial activity at significantly low MIC (0.3 ± 0.2 mg/ml) and MBC (0.7 ± 0.3 mg/ml) values in the broth medium according to the results of the microdilution test ($P < 0.001$), while MEE showed higher MIC (5.2 ± 3.8 mg/ml) and MBC (10.4 ± 7.6 mg/ml) values, which were comparable to ethyl alcohol MIC (6.0 ± 2.2 mg/ml) and MBC (12.0 ± 4.4 mg/ml) values. NaF showed no antibacterial activity, and chlorhexidine exhibited extremely low MIC and MBC values (9.7×10^{-4} and 4.7×10^{-4}, respectively).

Table 2. Antibacterial property of varnish ingredients against *S. mutans*

Varnishes	Antibacterial properties (mean ± SD)		
	Inhibition area (mm)	MIC (mg/ml)	MBC (mg/ml)
MEE	12.1 ± 1.2[1]	15.2 ± 3.8[1]	10.4 ± 7.6[1]
PEE	0	0.3 ± 0.2 [2]	0.7 ± 0.3 [2]
NaF	0	-	-
Ethyl alcohol	0	6.0 ± 2.2	12.0 ± 4.4
Chlorhexidine	17.0 ± 0.0	9.7×10^{-4}	4.7×10^{-4}

1 and 2 indicate significant differences as opposed to other groups ($P < 0.05$).

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3.3. Radiographic examination

Fig. 2 illustrates the dental density of the specimens in the acidic medium via radiography. The results are expressed in grayscale from 0 to 255. In general, the grayscale analysis shows that the density of the samples in all varnish preparations is similar, except for the non-treated group after the treatment period ($P < 0.01$).

3.4. Varnish buffering capacity

Fig. 3 displays the buffering capacity of the varnish films in the diluted acetic acid medium. Overall, the varnish films showed a great capacity for enhancing the medium pH back to neutral, except for the non-buffered varnish preparation.

Concerning the MEE/NaF V. preparation, the medium pH rose quickly and reached 7 at 3 min post-incubation. This trend is exactly reflected in the medium with PEE/NaF V, arriving at pH 7 at the same 3 min before reaching a plateau. The pH enhanced steadily and reached 7 for the NaF V at the end of the incubation period.

For the Preventa, pH increased up to 6 within 2 minutes of incubation that stood at the same value until the end of the incubation period, showing a significant difference compared to other preparations ($P < 0.01$). The acidity of the medium remained unchanged (at pH 4) from the beginning to the end of the incubation period in the control non-buffered varnish preparation.

3.5. Fluoride release

Fig. 4 and Table 1 show the concentration of fluoride ions released from the varnish films. All F-containing varnish films released their fluoride to almost the same extent, with some marginal differences ($P > 0.05$). The MEE/NaF V. preparation had the highest release of fluoride ions (92 ± 23). Following that, PEE/NaF V. and NaF V. showed exactly the same amount of F release (86 ± 18 and 86 ± 15, respectively), and Preventa released 77 µmoles of F on average (77 ± 12).

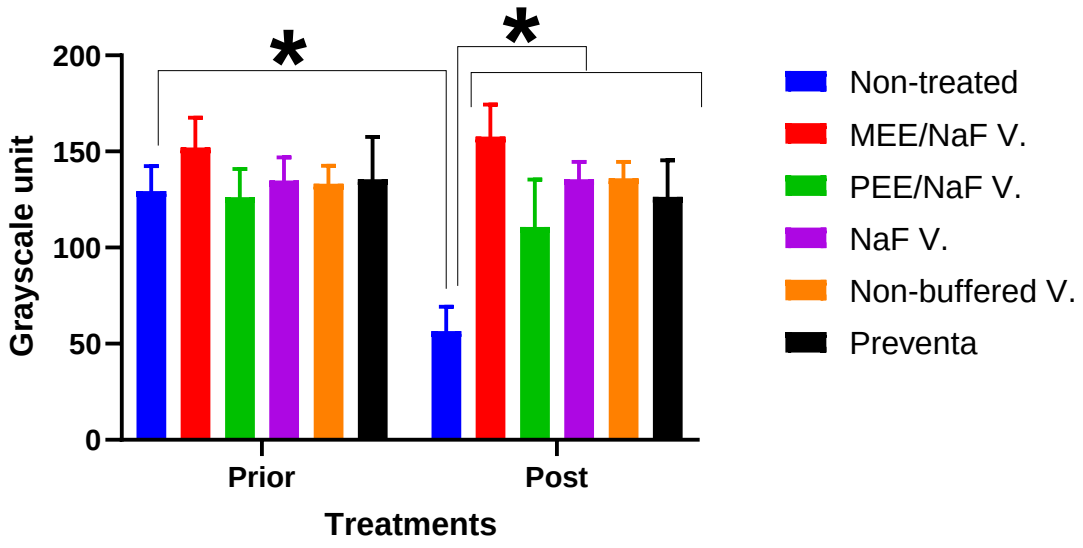

Fig. 2. Grayscale quantitative data related to the dental specimen's radiographs prior to and after the incubation period in a harsh acidic medium (pH = 2). Data are shown as mean ± standard deviation (n = 9). * indicates a significant difference between the groups ($P < 0.05$).

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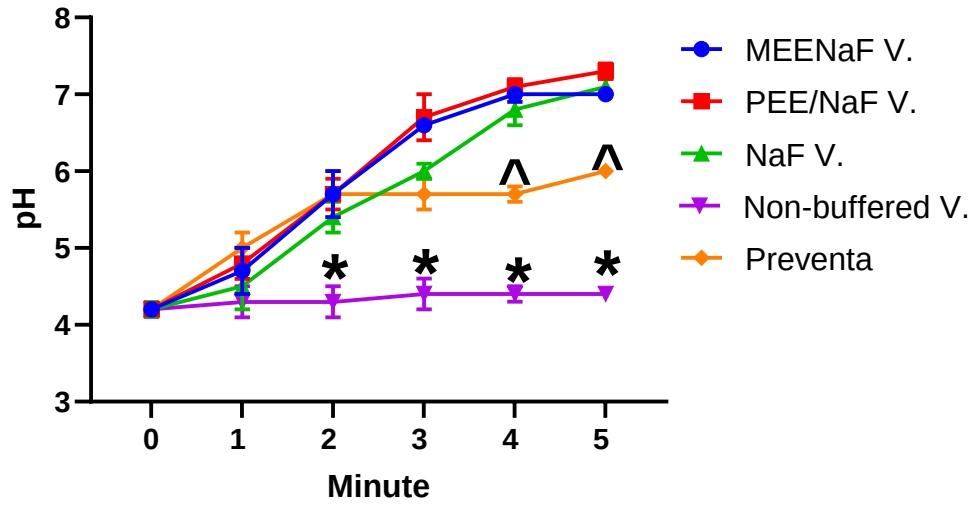

Fig. 3. The pH profile of the acetic acid medium in the wells covered with the varnish films within the 5-minute incubation period. Data are shown as mean ± standard deviation (n = 9). * and ^ indicate significant differences compared to non-buffered varnish and Preventa, respectively (P < 0.05).

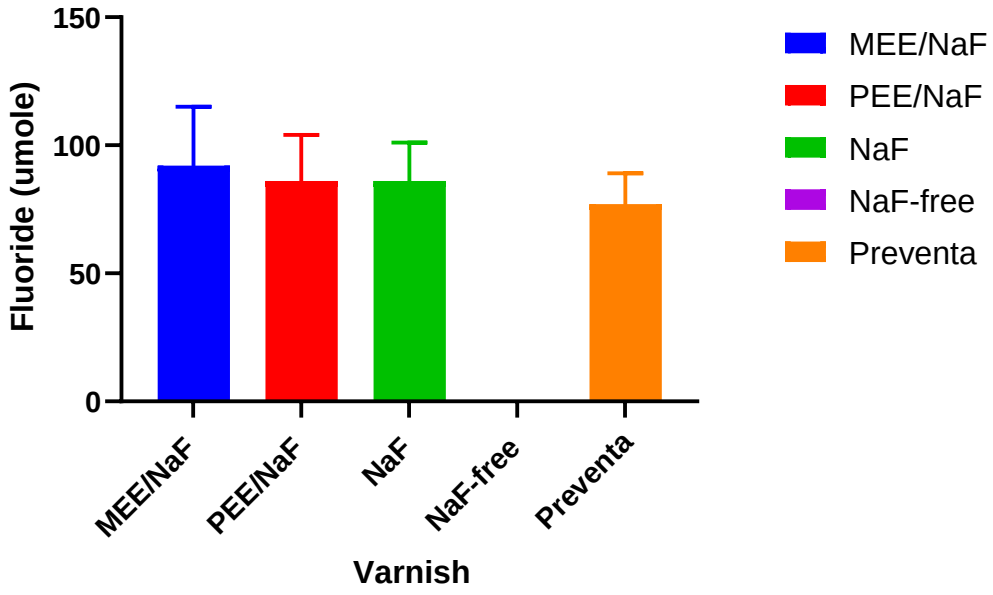

Fig. 4. The amount of fluoride released from varnish preparations in the acetic acid medium in terms of micromoles/ml varnish. Data are shown as mean ± standard deviation (n= 6).

4. Discussion

Previous investigations have revealed the anti-caries activity of PEE and MEE as ingredients in various preparations of varnishes [7, 13, 20, 21]. In these studies, the anti-caries potential of PEE was investigated in terms of *S. mutans* growth limitation, plaque establishment, scored dentinal caries, and calcium ion release [20, 21]. In contrast, the studies on the application of MEE were more limited, investigating mainly the antibacterial activity of MEE *in vitro* [7]. Although the pivotal role of

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acidity is well-known for caries progression [2], no study to our knowledge has so far directly investigated the buffering and protective capacity of varnish upon pH-drop challenge; as such, these questions spurred us into the present study.

It was revealed that MEE and PEE offer valuable antibacterial properties and are appropriate candidates to be applied in a varnish preparation. MEE and PEE both exhibited considerable antibacterial activity in the bacterial cultures of *S. mutans* (Table 2), which is known as the characteristic cariogenic microorganism in dentinal plaques. MEE per se displayed a large bacterial growth inhibition zone on the agar culture around its discs, and PEE illustrated outstanding antibacterial activity in the broth culture. MEE and PEE were also found to be effective antibacterial ingredients in the varnishes, leaving a considerable growth inhibition zone on the agar culture (Table 1).

The varnish consisted of additional ingredients with anti-cariogenic properties such as buffering capacity. All films made of buffered varnish were proven to quickly counter the pH-drop challenge in the acetic acid medium, which was highly surprising in our study and is also a crucial capacity for dental varnishes to have. Previous studies have shown that pH drops to 4 within a short period of glucose challenge in the dental plaque, where recurrent glucose challenges instigate demineralization of teeth to a clinical proportion in the long run [2]. The varnish's buffering capacity, as a result, counteracts the pH-drop challenge and reduces the rate of demineralization.

As opposed to the demineralization process, the lost mineral tissue is replaced with fresh healing tissue in the healing process known as demineralization [8, 19]. It is well known that the substitution of calcium with fluoride ions gives rise to the formation of fluorapatite, a mineral tissue resistant to dissolution in acidity [9]. During incubation of varnish films in the acidic medium, a considerable amount of fluoride ions was found to be released into the medium from the varnishes (average 85 µmoles), an amount of fluoride release that is comparable to those of previous investigations [19].

Above all, it was the prime protection of the dental tissues from the harsh acidic conditions when covered with the test varnishes, as revealed by the radiographic examinations [18]. Among the varnishes, there was no significant difference in terms of the final mineral density of the dental units. Coating the dental specimens with varnishes might provide an impermeable layer against the protonating medium that prevents the covered tooth from acidic dissolution. While the non-covered dental specimens had lost their entire mineral content, those coated with the varnishes withstood the harsh acidic conditions perfectly. We also attempted to simulate the plaque- and caries-promoting conditions with *S. mutans in vitro* in a high-glucose bacterial culture as per [22]; however, except for only short spells of mild pH drop to 5.5, we failed to observe any clinical evidence of dental demineralization on the non-treated dental tissues. Nonetheless, our radiographic examination explicitly demonstrated the outstanding protection of the varnish-covered teeth in the acidic environment.

5. Conclusions

Our investigation illustrated the role of every component of a typical varnish in a set of *in vitro* experiments. The varnish offered a noticeable antibacterial, buffering, and fluoride-releasing capacities against the caries-promoting

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conditions. It also protected the teeth from dissolution in the acidic medium. PEE and MEE had both effective antibacterial activity, which can decrease growth of *S. mutans* known to be involved in dental plaque establishment and caries progression. The studied features might translate to prolonged protection of teeth in clinical settings, and strongly supports applying varnishes for the long-term protection of teeth.

Conflict of Interest

We confirm that there is no conflict of interest. The manuscript does not contain any studies with human or animal subjects.

Author Contributions

A. D. and S. S. designed and collocated the data, P. Z. M. and S. M. collected and analyzed the data, and M. T. analyzed the data and draft the manuscript. All authors have revised and approved the final version of the manuscript.

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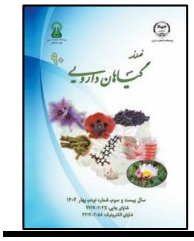

مقاله تحقیقاتی

ارزیابی اثر ضد پوسیدگی وارنیش حاوی گیاه دارویی مسواک (*Salvadora persica* L.) و بره موم بر روی دندان‌های پرمولار و مولار استخراج شده انسان

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اطلاعات مقاله

گل‌واژگان:
املاح‌زدایی دندان
فعالیت ضد باکتریایی
مسواک (*Salvadora persica*)
بره موم
وارنیش

چکیده

مقدمه: پوسیدگی دندان پیامد فعالیت میکروارگانیسم‌های پوسیدگی‌زا است که در پلاک دندان ساکن هستند. از این بین *Streptococcus mutans* عامل اصلی در ایجاد و پیشرفت تخریب بافت دندان است. **هدف:** در این مطالعه، فعالیت ضدپوسیدگی وارنیش برپایه ترکیبات طبیعی بر علیه *Streptococcus mutans* بر روی مجموعه‌ای از نمونه‌های دندانی ارزیابی شد. **روش بررسی:** ۵۴ نمونه دندان مولر و پرمولر انسان از یک کلینیک سرپایی دندانپزشکی کودکان در شهر بجنورد، ایران تهیه شدند. این دندان‌ها در یک بتونه سیلیکونی قالب‌گیری شده و با وارنیشی متشکل از عصاره‌های اتانولی بره موم و مسواک (از شاخه‌های درخت *Salvadora persica*) تیمار شدند و در محیط اسیدی (pH 2) انکوبه شدند. در مرحله بعد، تراکم نمونه‌ها با استفاده از رادیوگرافی، فعالیت ضد باکتریایی با استفاده از تست‌های انتشار دیسک و میکرودیلوشن و ظرفیت بافری و آزادسازی فلوراید توسط چالش قطره‌ای pH در محلول اسید استیک مورد بررسی قرار گرفت. **نتایج:** به طور کلی، نتایج بررسی‌های رادیوگرافی نشان داد که وارنیش‌ها نمونه‌های دندانی را از انحلال در محیط اسیدی محافظت می‌کنند. همچنین، وارنیش فعالیت ضد باکتریایی قابل توجهی از خود نشان داد. علاوه براین، وارنیش ظرفیت بافری قابل توجه (افزایش pH از ۴ به ۷ در عرض ۵ دقیقه) و قابلیت آزادسازی محتوای بالایی از فلوراید (میانگین ۸۵ میکرومول) را نشان داد. **نتیجه‌گیری:** استفاده از وارنیش بر پایه ترکیبات طبیعی مسواک و بره موم برای محافظت از دندان به شدت توصیه می‌شود.

مخففها: MEE، عصاره اتانولی مسواک؛ PEE، عصاره اتانولی بره موم

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