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Comprehensive evaluation of early shear bond strength and antimicrobial activity in orthodontic adhesives enhanced with salvadora persica oil

Amir Abdulhadi, Sarmad S. Salih Al Qassar and Ahmed Mudhafar Mohammed[1]

Abstract

OBJECTIVES: This study investigates the mechanical properties and antimicrobial efficiency of orthodontic adhesive modified with Salvadora persica (SP) oil, including adhesive remnant index (ARI) and shear bond strength (SBS), specifically antimicrobial efficacy against *Streptococcus mutans*.

METHODS: Forty freshly extracted human premolars were recruited. They were classified into four groups according to the concentration of SP oil added to Heliosit orthodontic adhesive where the control group was with no adhesive modification, alongside three experimental groups, wherein SP oil was integrated into the adhesive at concentrations of 1%, 3%, and 5% weight/weight, respectively. The tooth buccal surface was etched by phosphoric acid gel (37%). The orthodontic brackets utilized were standard stainless steel edgewise 22". The brackets were bonded with Heliosit by Woodpecker LED light cure for 20 sec. The SBS was assessed using a universal testing machine, and ARI was inspected by a stereomicroscope at 20X magnification power. The antimicrobial activity against Streptococcus mutans was evaluated. The statistical analyses, analysis of variance (ANOVA), and Kruskal-Wallis and Duncan were performed where $P \leq 0.05$.

RESULTS: The findings indicated that among the experimental groups, the 3% SP oil group exhibited the highest mean SBS value, following closely behind the control group. Conversely, the mean SBS was lowest for the SP group with a 5% concentration. However, ANOVA and Kruskal-Wallis tests revealed no significant differences between groups ($P \geq 0.275, 0.069$), respectively. Antimicrobial tests demonstrated a concentration-dependent antibacterial effect, the 5% group exhibiting the highest efficacy.

CONCLUSION: Orthodontic adhesive modified with SP oil maintains favorable SBS while demonstrating antimicrobial effects against Streptococcus mutans.

Keywords:

Adhesive remnant index, antibacterial activity, Salvadora persica oil, shear bond strength

Department of Pedodontics, Orthodontics and Preventive Dentistry, College of Dentistry, University of Mosul, Mosul, [1]Department of Chemistry, College of Education for Girls, University of Mosul, Mosul, Iraq

Address for correspondence:
Dr. Sarmad S. Salih Al Qassar, Department of Pedodontics, Orthodontics and Preventive Dentistry, College of Dentistry, University of Mosul, Iraq.
E-mail: sarmadsobhi@uomosul.edu.iq

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Introduction

Orthodontic treatment's primary goal is repositioning misaligned teeth correctly in the dental arch and reshaping the supporting gum and bone tissues through the application of orthodontic force.[1]

Orthodontic fixed appliance including attachments on the tooth surfaces can impede the maintenance of oral hygiene, resulting in the growth of complex biofilms composed of cariogenic bacteria and buildup of plaque. In certain instances, this situation may necessitate rescheduling the treatment.[2] Subsequently, demineralization may occur around orthodontic attachments (brackets, bands)

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as a result of the presence and activities of diverse bacterial species. Notably, primary oral pathogens, such as *Lactobacillus acidophilus* and *Streptococcus mutans*, play pivotal roles in the development of dental plaque. *Streptococcus mutans* is recognized as a primary contributor to the initial demineralization process of dental hard tissues, whereas it was identified as a significant factor in white spot lesions (WSLs), particularly in the absence of specific preventive measures.[3] The possible adverse consequences that may arise during orthodontic treatment encompass the generation of WSLs and the initiation of early-stage dental decay in regions adjacent to bonded orthodontic attachments.[4] Nevertheless, it is advisable to utilize primary preventive strategies, such as preserving excellent oral hygiene and adhering to a low-sugar diet, alongside secondary prevention techniques, such as applying fluoride, to avert these potential side effects. However, these approaches for preventing WSLs may not be entirely dependable due to their reliance on individual cooperation.[5] In recent years, different antimicrobial nanoparticles were incorporated into the orthodontic adhesive to increase their efficiency against the microorganisms correlated to the formation of WSLs. However, the efficiency of this strategy has been questioned because of the restricted short-term antimicrobial impacts and the inadequate mechanical characteristics observed in modified adhesives containing antimicrobial agents.[6]

In recent years, there has been a conspicuous trend rise in worldwide recognition of the utilization of herbal remedies for addressing diverse health issues. The heightened awareness stems from the favorable outcomes observed in herbal therapies, coupled with their limited adverse effects.[7]

Among the plant treatments supported by evidence, Salvadora persica (SP) is highly regarded and sometimes referred to as a "miracle twig."[8] SP, often recognized as Miswak, belongs to the Salvadoraceae plant family.[9] Its principal geographical distribution encompasses arid and subtropical regions within the Middle East and Africa.[10]

The roots, branches, and fresh leaves of the tree can be incorporated into daily dietary habits and have been traditionally used in herbal remedies for conditions, such as coughs, asthma, scurvy, oral hygiene, and various purposes.[11]

The positive impacts of SP roots on dental health are a result of both its physical cleaning action when employed for brushing and its pharmacologically active constituents. These include chemical substances, such as tannins, which hinder the glucosyltransferase enzyme, leading to a decrease in plaque formation and periodontal diseases, as well as its oil that offer defense against tooth decay and plaque formation.[12]

A multitude of inquiries have inspected the influence of miswak on oral health, including using the SP stick as toothbrush[13] and also using SP as anti-biofilm against *Streptococcus Mutans*.[14] Some studies were about the antibacterial activities of SP extracts.[15] Also, Halawany *et al*.[16] studied the antimicrobial effectiveness of SP extract on monospecies biofilm formation on orthodontic brackets.

To our current knowledge, there is a dearth of research dedicated to evaluating the influence of integrating SP essential oil into orthodontic composite materials, which are conventionally utilized for bracket bonding in the context of orthodontic interventions. Thus, this study endeavors to assess the impact of incorporating SP oil at varying concentrations on adhesive remnant index (ARI) and the shear bond strength (SBS) of orthodontic adhesives, while concurrently investigating the antibacterial effectiveness of SP oil-modified orthodontic adhesive against *Streptococcus mutans*. The null hypothesis of the study includes no significant difference in ARI and SBS of orthodontic adhesive for bonded orthodontic brackets after the incorporation of different concentrations of SP oil. Also, there is no effect of the incorporation of different concentrations of SP oil on the orthodontic adhesive on the *Streptococcus mutans*.

Materials and Methods

The Ethics Review Board of the College of Dentistry, University of Mosul, granted ethical clearance in 2023 (UoX.Dent/H.DM. 51/22).

The criteria for tooth selection involved ensuring teeth were free of hypo-plastic areas, caries, attrition, cracks, gross irregularities, and restorations. This was checked using a stereomicroscope (Optima, China) at 10 X magnification power. Additionally, selected teeth had not undergone orthodontic or endodontic therapy and had no previous treatment with chemical substances, such as formalin, alcohol, or H_2O_2.[6]

The sample size calculation followed the formula: $N = [(4\sigma^2) (Z_\alpha + Z_\beta)^2] \div E^2$

where N: is the number of experimental samples,

σ: is the assumed standard deviation, it was = 2.23.[17]

Z_α =1.96 for a = 0.05 (two-tailed),

Z_β =0.80 for the 80% power

E is the detectable difference between treatment means = 4.

Accordingly, the sample size estimation was conducted with 10 teeth for each study group, regarding the SBS test and ARI, while the sample size for the antimicrobial test was 3 coordinated with previously published researches.[18]

In relevant to the above formula, the study samples comprised 40 human premolar teeth (first and/or second premolars) that were extracted for the orthodontic purpose from patients seeking orthodontic treatment, where their age ranges between 16 and 25 years old, and they were obtained from private clinics and Dental Health Centers at the city of Mosul/ Iraq. The teeth were washed and stored in a dark container with distilled water, and taking measures to avoid dehydration might adversely impact both dentin moisture levels and bond strength,[19] by storing teeth at room temperature (20–25°C). The allocation of teeth into four groups was conducted randomly in accordance with the study's design.

Mixing SP oil to adhesive

The SP oil was obtained from Atariya School in Jeddah/ Saudi Arabia, and the oil was preserved in special dark container to prevent any interaction with air or light.[20] The orthodontic adhesive used in this research was Heliosit, Ivoclar, Zurich, Switzerland). The oil was added to the Heliosit adhesive at a specific percentage using sensitive four-digit weight scaler (Kern, Germany) and mixed well manually using dappen dish with mortar in a semi-dark place at room temperature (25°C), and the resulting mixture was then placed in dark container to prevent light-emitting.

The oil addition was conducted as below:

1- No oil was added to the adhesive which acts as a control group (0%).
2- Addition of 1 mg SP oil to 99 mg of Heliosit adhesive represents a 1% group.
3- Addition of 3 mg of SP oil to 97 mg of Heliosit adhesive represents a 3% group.
4- Addition of 5 mg of SP oil to 95 mg of Heliosit adhesive represents a 5% group.

Grouping of the samples

The forty teeth were categorized into four groups as shown in Figure 1:

A—10 teeth as a control group which were not subjected to any addition of the SP oil.
B—10 teeth were used with the addition of 1% of SP oil to the adhesive.
C—10 teeth were used with the addition of 3% of SP oil to the adhesive.
D—10 teeth were used with the addition of 5% of SP oil to the adhesive.

The teeth utilized in this study were immobilized by securing their roots within a substance created through the application of cold-cure acrylic, encased by a plastic mold. The buccal surfaces were aligned parallel to the base, employing a dental surveyor to ascertain uniform parallel alignment and mitigate any potential aberrations in the results.

The brackets used in this research were made from Dentaurum Company, Germany, standard edgewise system (premolar brackets) (ultra-minitrim) 0.22".

The teeth underwent cleansing and polishing with pumice and rubber prophylactic cups for a duration of 10 seconds before bonding,[21] then dried, underwent etching by 37% phosphoric acid (Total Etch, Ivoclar Vivadent, Liechtenstein) for 30 seconds,[22,23] then rinsed gently using distilled water, and dried by air using a

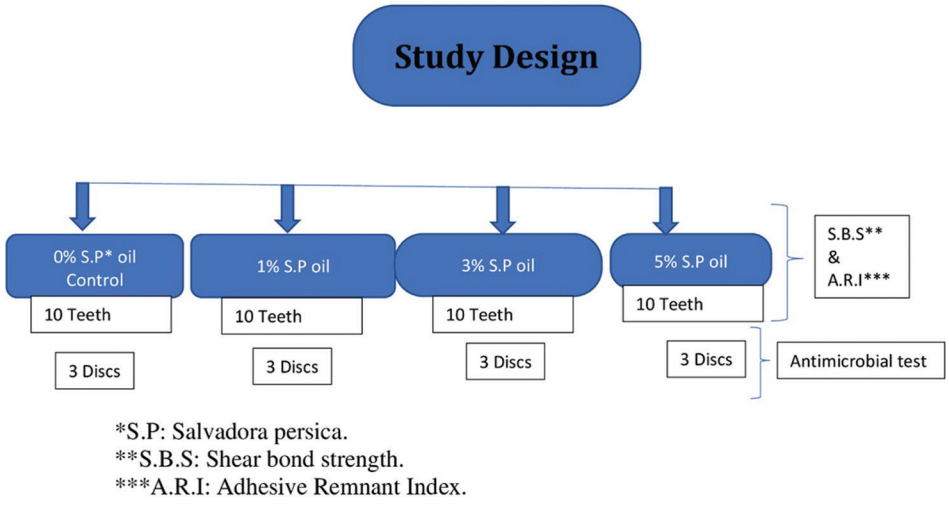

Figure 1: Diagram illustrates the design of the study

triple syringe; then, the adhesive was added to bracket base in accordance with the manufacturer's instructions. The bracket was adjusted to the crown by bracket positioner (Dentaurum, Germany).

The surface of the enamel hosted the attachment of the bracket. A universal material testing machine applied the predetermined force (100 gm) for secure uniform placement and adhesive thickness. The excess resin was removed by the sharp probe. Subsequently, a dental office-acquired light-emitting diode (LED) (Woodpecker, China) with a 440 nm wavelength was employed to light cure the adhesive for 20 seconds,[24] ensuring comprehensive curing from mesial and distal proximal margins (10 sec each). After attaching brackets, the teeth were positioned within dark incubators containing distilled water for a duration of 24 hours before undergoing the SBS test.

SBS

The SBS, quantified in newtons, was ascertained by employing a universal testing machine from Gester Instruments Co. in Fujian, PR China. The testing was conducted at a rate of 0.5 mm/min.[25]

All the records were divided by the area of the premolar internal bracket base area (bonding area) with curvature (10.03 mm^2) (according to the manufacturer's specifications) to convert it to megapascal.

ARI

The teeth that underwent debonding were scrutinized at a magnification power of 20X utilizing a stereomicroscope (Optika, Italy) to determine the extent of adhesive residue present on the buccal surface of the teeth, employing the ARI, and its associated scoring system was as follows:

- 0: Reflects no presence of composite remnants on the enamel surface.
- 1: Represents less than 50% of the composite remaining on the surface of the enamel.
- 2: Indicates over 50% of the composite remaining on the surface of the enamel.
- 3: Signifies the complete retention of the composite on the surface of the enamel, along with a visible impression of the bracket base on the remaining composite.

Antimicrobial test

Three specimens of disks were created for each group using translucent plastic molds.

The disks were 3 mm in diameter and 2 mm thick. Following the filling of the molds with composite, they were enveloped with celluloid matrix strips and subjected to light curing [(LED (Woodpecker, China) with a 440 nm wavelength)] for a duration of 20 seconds; the light curing was performed from the top of the mold and another exposure was directed from the opposite side to ensure polymerization. Following this, the disks were extracted from the mold after solidifying. To sterilize the disks, they were immersed in 70% alcohol for 30 minutes at room temperature. A sterilization check was performed by incubating one disk in broth media for 24 hours, showing no growth.[22,23]

In accordance with the manufacturer's guidelines, Mueller-Hinton medium plates were prepared, and standardization inoculation was conducted.

The Kirby-Bauer test using Mueller-Hinton agar included the application of *Streptococcus mutans* with a swab onto the plate, allowing it to dry and placing tested disks on the agar surface. The plates underwent a 48-hour incubation period at 37°C under anaerobic conditions, after which the resulting inhibition zones were quantified and expressed in millimeters.

Three Petri dishes were used in total, each implanted with three disks (CG, 1% SPOG, 3% SPOG, and 5% SPOG).

The statistical analysis was conducted using Windows programs, including the Statistical Package for the Social Sciences (SPSS) version 26. The Shapiro-Wilk test was used for the estimation of the normal distribution of the data, while descriptive statistics (mean and standard deviations) were performed to account for the results. For further inspection of the data, analysis of variance (ANOVA), and Kruskal and Duncan tests were performed (where $P \geq 0.05$).

Results

SBS

The analysis of data normality within groups (Shapiro-Wilk test) indicates that SBS data follow a normal distribution, as illustrated in Table 1.

Statistical analysis of SBS

Table 2 presents descriptive statistics for SBS, encompassing mean values, standard deviation, across study groups. The analysis of the initial bonded groups indicates that the control group exhibits the highest mean SBS (8.47) Mpas, followed by the orthodontic adhesive modified with 3% of SP essential oil (1% follows closely), while the 5% group demonstrates the lowest mean value.

The outcomes of the one-way ANOVA statistical test are presented in Table 2, indicating no significant difference where P is 0.275 and P value was adjusted to be ($P \geq 0.05$) for the various groups in this study.

Statistical analysis of ARI

The ARI test data that were collected samples were enrolled in SPSS for score frequency evaluation [Table 3] that were analyzed by the Kruskal-Wallis test for all groups; the resultant *P* value was 0.069, which indicates a non-significant difference between groups.

Antimicrobial results

Figure 2 illustrates the results of antibacterial activity at various concentrations, which shows that an increase in SP oil addition to the adhesive has high antimicrobial resistance in contrast to low concentrations of SP oil addition ranging from 13 to 17 mm inhibition zone, when compared to the control group (zero SP oil) which illustrated zero antimicrobial efficiency against *S. mutans*. Table 4 illustrates $P \leq 0.001$ which designates that there are significant differences between studied groups assessed by ANOVA. Also, Table 4 shows the antibacterial homogeneous subsets with the Duncan test.

Discussion

Previous studies have suggested that a higher occurrence of cariogenic bacteria, including *S. mutans* species within the dental biofilm around brackets, might be linked to enamel decalcification and the initiation of WSLs and initial caries in individuals undergoing orthodontic treatment.[26,27] In essence, the components integrated should possess robust capabilities in hindering the growth and colonization of cariogenic bacteria, with a preference for promoting enamel remineralization.[28,29]

In this study, SP oil, acknowledged for its potential as an effective anti-caries agent, was utilized to amend orthodontic bonding material. SP demonstrates a multifaceted spectrum of biological attributes, encompassing antiviral and antibacterial properties.[30]

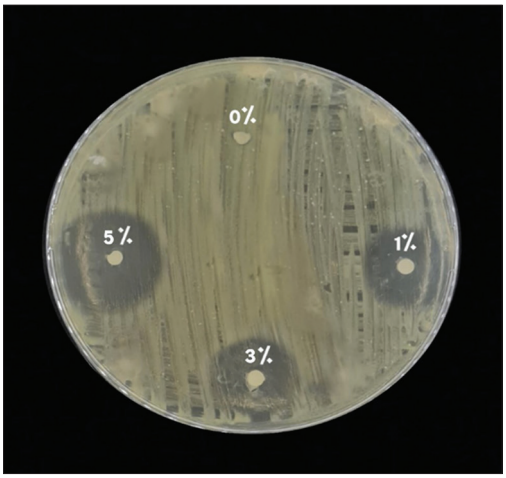

Figure 2: Antibacterial activity of tested materials against *S. mutans*

This study was performed to search the effect of the incorporation of different percentages of the SP oil to the orthodontic adhesive, as SP extract was proved to have an impact against antimicrobial oral microorganism.[7,8,23] This study at least according to our knowledge was the first study performed on SP oil in the orthodontic adhesive.

The methodology of this study followed the previously published research dealing with modified orthodontic composite regarding the SBS and antimicrobial efficiency. Sample collection and storage, adhesive oil mixing, bonding protocol, debonding tests, and antimicrobial tests were coordinated with previously approved procedure.[8,21,31]

The SBS of the Heliosit orthodontic adhesive in the control group was 8.7 Mpas, which represent the acceptable

Table 1: Shapiro-Wilk test of data distribution along SBS-bonding groups

Variable	Statistic	*P**
Control	0.877	0.149
Modified with 1% SP	0.927	0.459
Modified with 3% SP	0.953	0.731
Modified with 5% SP	0.917	0.372

*Not significant at $P \geq 0.05$

Table 2: Descriptive statistics for the SBS of bonded groups

Variable	Mean	SD	*P**
Control	8.473	4.802	0.275
Modified with 1% SP oil	5.711	3.430	
Modified with 3% SP oil	6.562	2.858	
Modified with 5% SP oil	5.697	2.310	

SD is the standard deviation, SP is the Salvadora persica essential oil, SBS measurement unit is MPa. *Not significant at $P \geq 0.05$

Table 3 ARI scores' frequencies and Kruskal-Wallis test of adhesive remnant index (ARI)

Modified adhesive	ARI scores				*P**
	0	1	2	3	
Control 0%	1	4	4	1	0.0694
Modified with 1% SP** oil	1	3	6	0	
Modified with 1% SP oil	1	0	5	4	
Modified with 1% SP oil	2	3	4	1	

*Kruskal-Wallis test, $P \geq 0.05$. **SP represents Salvadora persica

Table 4: Descriptive analysis, ANOVA, and Duncan statistical tests of antimicrobial inhibition zone

Sample groups	*n**	Mean (SD) mm	**P
Control	3	0000 (00000)[a]	0.001
Modified with 1% SP* oil	3	13.1667 (0.29)[b]	
Modified with 3% SP* oil	3	15.0000 (1.0)[c]	
Modified with 5% SP* oil	3	17.3333 (0.578)[d]	

**n* represents the number of disks prepared for the tests. **ANOVA test. Groups marked with different letters (uppercase) validate significantly different outcomes of the Duncan test, where $P \leq 0.05$

range of SBS according to the requirement of the orthodontic adhesive for clinical practice.[2] Also, it can withstand the chewing force safely. Alqassar *et al*. in 2023 gained 8.5 Mpas SBS using the same adhesive; this could validate the methodological protocol conducted in this study as well as subsequent outcomes of SP oil addition. In SBS, analysis of the bonded groups indicates that there were no significant differences between groups, as the control group exhibits the highest mean SBS which was the same as researches conducted before,[2,32,33] followed by the orthodontic adhesive modified with 3% of SP essential oil (1% follows closely), while the 5% group demonstrates the lowest mean value. However, the recorded values at 3% SP oil are within the acceptable limits of shear strength.

No significant differences between groups according to the Kruskal-Willis test of ARI data represent the homogeneity between the orthodontic adhesive used in this study with SP oil. This could be related to the harmony induced between the polymer backbone with the component of the SP oil.

While the antibacterial efficacy of SP may appear conventional in comparison with alternative natural products, its remarkable capacity to modulate the equilibrium between enamel demineralization and remineralization distinguishes it, positioning SP as a notable contender among diverse anti-caries natural agents.[31] The advantage of the SP oil incorporation could not only hinder the growth, adherence, and acid production of specific cariogenic bacteria but also possess the capability to maintain the equilibrium between enamel demineralization and remineralization.[30-34]

In summary, orthodontic adhesive containing SP oil demonstrates a potential inhibitory impact on the growth and adhesion of *S. mutans*. without compromising bond strength.

Clinically, this study presents a novel approach to enhancing orthodontic adhesives through the incorporation of SP oil, known for its antimicrobial properties. The modified adhesive demonstrates favorable SBS while exhibiting advantageous antimicrobial effects.

The identified constraints offer avenues for future investigations and improvements in the development of orthodontic adhesives with enhanced antimicrobial properties.

Clinical implication

Orthodontic clinicians can modify their adhesive by a specific percentage of SP oil to prevent WSLs that follows long-term orthodontic treatments.

Study limitations

A primary constraint inherent in this study could be associated with the exclusive use of a single type of orthodontic composite besides that the effect of thermocycling was also not investigated.

Conclusions

SP oil can be added at different concentrations up to 5% concentration to orthodontic adhesive which has no significant effect on SBS with upright antibacterial activity against *Streptococcus mutans* subsequently reducing the incidence of WSL formation.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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