Title assessment of the mechanical properties and antimicrobial efficiency of orthodontic adhesive modified with Salvadora Persica oil

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Title assessment of the mechanical properties and antimicrobial efficiency of orthodontic adhesive modified with Salvadora Persica oil

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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), shear bond strength (SBS), and assess its antimicrobial efficacy against *Streptococcus Mutans*.

Methods. 40 freshly extracted human premolars were recruited. These teeth underwent thorough cleaning, polishing, and rinsing with water before being dried. Subsequently, the buccal surface was etched using phosphoric acid gel (37%). The orthodontic brackets utilized were standard stainless steel. The brackets were bonded using (Heliosit, ivoclar, Zurich, Switzerland) orthodontic adhesive in four distinct groups: a control group was established wherein bonding occurred without any adhesive modification, alongside three experimental groups wherein Salvadora persica oil was integrated into the adhesive at concentrations of 1%, 3%, and 5% weight/weight, respectively. The SBS was then assessed using a universal testing machine and antimicrobial activity against Streptococcus Mutans were assessed. Statistical analyses, including Kruskal-Wallis and one-way ANOVA tests, were performed.
Results. The findings indicated that among the experimental groups, the 3% concentration of Salvadora persica oil exhibited the highest mean shear bond strength value, following closely behind the control group. Conversely, the mean shear bond strength values were lowest for the SP group with a 5% concentration. ANOVA revealed no significant differences either within or between the mean values ($p \geq 0.05$). Antimicrobial tests demonstrated a concentration-dependent antibacterial effect, with 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.

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 attachments can impede the maintenance of oral hygiene, resulting in the growth of complex biofilms composed of cariogenic bacteria and the buildup of plaque. In certain instances, this situation may necessitate rescheduling the treatment [2].

During orthodontic treatment, demineralization may occur around orthodontic attachments (brackets, bands, ...) as a result of the presence and activities of diverse bacterial species. Notably, primary oral pathogens such as *Lactobacillus acidophilus* and *Streptococcus mutans*, which play pivotal roles in the development of dental plaque. *S. mutans* is recognized as a primary contributor to the initial demineralization process of dental hard tissues, whereas *L. acidophilus* is identified as a significant factor in the progression of caries, particularly in the absence of specific preventive measures [3]. The possible adverse consequences that may arise during orthodontic treatment encompass generation of white spot lesions (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 like applying fluoride, to avert these potential side effects. However, these approaches for preventing tooth decay may not be entirely dependable due to their reliance on individual cooperation [5]. 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 traditional antimicrobial agents [6].

In recent times, 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, belon... to the Salvadoracea 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 scrutinized the influence of miswak on oral health, including using the SP stick as tooth brush [13]. Also using SP as anti-biofilm against *Streptococcus Mutans* [14], some studies were about antibacterial activities of SP extracts [15]. Also [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.

Aims of the Study

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*.

Hypothesis of the Study

1. There is a significant difference in adhesive remnant index (ARI) and Shear Bond Strength (SBS) of orthodontic adhesive for bonded orthodontic brackets after incorporation of different concentrations of SP oil.
2. There is no effect of 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 xxxx granted ethical clearance in 2023 (UoX.Dent/H.DM. 51/22).
The criteria for teeth selection involved ensuring teeth were free of hypo-plastic areas, caries, attrition, cracks, gross irregularities, and restorations. This was achieved by using stereomicroscope (Optima, China) at magnification 10 x. Additionally, selected teeth had not undergone orthodontic or endodontic therapy and had no previous treat with chemical substances such as formalin, alcohol or H_20_2 [17].
The sample size calculation followed the formula: $N= [(4\sigma^2) (Z_\alpha + Z_\beta)^2] \div E^2$
N: is the number of experimental samples,
σ: is the assumed standard deviation, it was =2.23 [18].
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 10 teeth for each study group, regarding the SBS test [19].

In relevant to above formula, the study samples comprised 40 human premolar teeth (first and/or second premolars) that are extracted for orthodontic purpose from patients age between 16-25 years old, they were obtained from certain private clinics and Dental Health Centers the city of xxxx. Then teeth were washed and stored in a dark container with distilled water, taking measures to avoid dehydration might adversely impact both dentin moisture levels and bond strength [20], storing teeth at room temperature (20-25 C̊). 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 Shmool Jeddah / Saudi Arabia, the oil was preserved in special dark container to prevent any interaction with air or light [21]. The orthodontic adhesive used in this research was (Heliosit, ivoclar, Zurich, Switzerland). The oil was added to the Heliosit adhesive at specific percentage by using sensitive four digits weights scaler

(Kern, Germany) and well mixed manually using dappen dish with mortar in a dark place at room temperature (25 °C) then placed in dark container to prevent light emitting.
The addition was as bellow:

1- No oil was added to adhesive (control group 0%)
2- Addition of 1 mg SP oil to 99 mg of Heliosit adhesive (1%)
3- Addition of 3 mg of SP oil to 97 mg of Heliosit adhesive (3%)
4- Addition of 5 mg of SP oil to 95 mg of Heliosit adhesive (5%)

Grouping of the samples:

The forty teeth were categorized into four groups as shows in figure (1):

A-10 teeth as a control group which were not subjected to any addition of the materials.

B-10 teeth were used with addition of 1% of SP oil to adhesive.

C-10 teeth were used with addition of 3% of SP oil to adhesive.

D-10 teeth were used with addition of 5% of SP oil to adhesive.

The teeth utilized in this study were immobilized by securing their roots within a foundation 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 edge wise system (premolars brackets) (ultra-minitrim).

The teeth underwent cleansing and polishing with pumice and rubber prophylactic cups for a duration of 10 seconds [22], then dried and undergo etching by 37% phosphoric acid (Total etch, Ivoclar Vivadent, Liechtenstein) [23] for 30 seconds [24], then rinsed gently using distilled water and dried by air using triple syringe, then the adhesive added to bracket base accordance with the manufacturers' instructions. The bracket adjusted to the crown by bracket positioner (Dentaurum, Germany).

The surface of enamel hosted the attachment of the bracket. Following the application of the specified force for secure placement and the removal of excess resin, a universal material testing machine applied the predetermined force (100 gm). A pointed crosshead, operating at 0.5 mm/minute speed, facilitated this process. Upon reaching the predetermined bonding force, the pressure on the bracket was automatically relieved. 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 [25], ensuring comprehensive curing from all proximal margins. After attaching brackets, the teeth were positioned within dark incubators containing distilled water for a duration of 24 hours prior to undergoing the Shear Bond Strength (SBS) test.

Shear Bond Strength

The shear bond strength, quantified in newtons, was ascertained employing a universal testing machine from Gester Instruments Co. in Fujian, PR China. The testing was conducted in rate of 0.5 mm/min [26].

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 manufacturer specifications) to convert it to MegaPascal.

ARI (Adhesive Remnant Index)

The teeth that underwent debonding were scrutinized at a magnification power of x10 utilizing a stereomicroscope (Optika, Italy) to determine the extent of adhesive residue present on the buccal surface of the teeth, employing the Adhesive Remnant Index (ARI) and its associated scoring system.

» 0: Reflects no presence of composite remnants on the enamel surface.
» 1: Represents less than 50% of the composite remaining on the surface of enamel.
» 2: Indicates over 50% of the composite remaining on the surface of enamel.
» 3: Signifies the complete retention of the composite on the surface of 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 3mm in diameter and 2mm thick. Following the filling of the molds with composite, they were enveloped with celluloid matrix strips and subjected to light curing. (Radii plus, SDI, Victoria, Australia) 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.

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.

Six Petri dishes were used in total, each implanted with three disks (CG, 1% SPOG, 3% SPOG, and 5% SPOG). Statistical analysis was conducted using Windows programs, including SPSS (ANOVA, Duncan tests) and Microsoft Office 365 (Excel).

Results:

Shear Bond Strength

The analysis of data normality within groups indicates that most of the data follows a normal distribution, as illustrated in table (1).

Descriptive Analysis of SBS Groups

Table (2) presents descriptive statistics for SBS, encompassing mean values, standard deviation, as well as maximum and minimum values across study groups. Analysis of the initial bonded groups indicates that the control group exhibits the highest mean SBS, 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 analysis of variance (ANOVA) statistical test are presented in Table (3), indicating no significant difference,

($P \geq 0.05$) among the mean SBS values for the various groups in this study.

Adhesive Remnant Index (ARI)

The data that were collected from ARI tests were inserted in SPSS app for descriptive statics Table (4) then were analyzed using Kruskal-Wallis's test for all groups (table 5), which shows that $P \geq 0.05$ that means there is no significant differences between groups.

Antimicrobial results:

The figure (1) illustrates the results of antibacterial activity at various concentrations, which shows that increase in SP oil addition to adhesive has high antimicrobial resistance in contrast to low concentrations of SP oil addition. Table (6) illustrates $P \leq 0.05$ which means that there are significant differences between groups. Table (7) shows the antibacterial homogeneous Subsets (Duncan test).

Discussion

Previous studies have suggested that a higher occurrence of cariogenic bacteria, including *S. mutans* and *Lactobacillus* species within the dental biofilm around brackets, might be linked to enamel decalcification and the initiation of initial caries in individuals undergoing orthodontic treatment.[27 28]. 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 [29,30].

While the antibacterial efficacy of SP may appear conventional in comparison to 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].

In the present study, Salvadora persica (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 [32].

SP not only hinders the growth, adherence, and acid production of specific cariogenic bacteria but also possesses the capability to maintain the equilibrium between enamel demineralization and remineralization [31-34].

The incorporation of Salvadora persica (SP) oil was carried out manually at specific concentrations (0%, 1%, 3%, 5%), ensuring thorough mixing until a homogeneous mixture was achieved. Subsequently, the brackets were affixed to the teeth as per protocol and subjected to Shear Bond Strength (SBS) testing using a universal testing machine. Additionally, the Adhesive Remnant Index (ARI) was evaluated using a stereomicroscope (Optika, Italy) at a magnification of x10. Furthermore, three discs from each group of modified adhesives were prepared and assessed for their resistance against antimicrobial activity targeting *Streptococcus mutans*.

In SBS Analysis of the initial bonded groups indicates that there were no significant differences between groups, as the control group exhibits the highest mean SBS which were same as researches done before [33,34], followed by the orthodontic adhesive modified with 3% of SP essential oil (1% follows closely), while the 5% group demonstrates the lowest mean value. Authors still think that the recorded values at 3% SP oil are within the acceptable limits of shear strength, the statical results (Kruskal-Willis) of ARI data showed that there were no significant differences between groups.

Based on the findings from the antimicrobial test in this study, it can be inferred that the oil extract of SP exhibits strong antibacterial and antibiofilm activities against *Streptococcus Mutans* species, in which 5% modified adhesive has more antimicrobial effect than 3% followed by 1%.

In summary, orthodontic adhesive containing SP oil demonstrates a potential inhibitory impact on the growth and adhesion of *S. mutans*. Without compromising bond strength. 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 specific percentage of SP oil to prevent WSL (White Spot Lesion) 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, as well as the study needed more tests including FTIR (Fourier Transform Infrared) in which to find out the amount of leaching of oil after curing.

Conclusions: SP oil can be added at different concentration up to 5% concentration to orthodontic adhesive which has no significant effect on SBS and has good anti-bacterial activity against *Streptococcus Mutans* which will prevent accumulation of plaque and reducing the incidence of white spot lesion formation.

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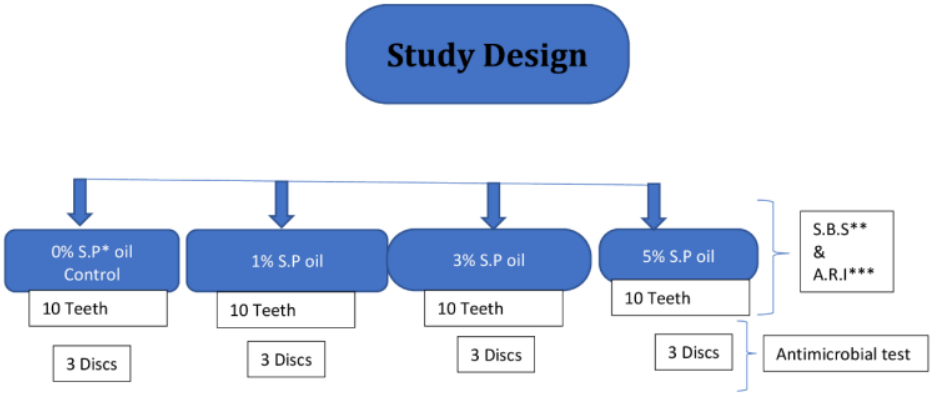

*S.P: Salvadora persica.
**S.B.S: Shear bond strength.
***A.R.I: Adhesive Remnant Index.

Figure (1) Diagram Illustrate the Design of the Study

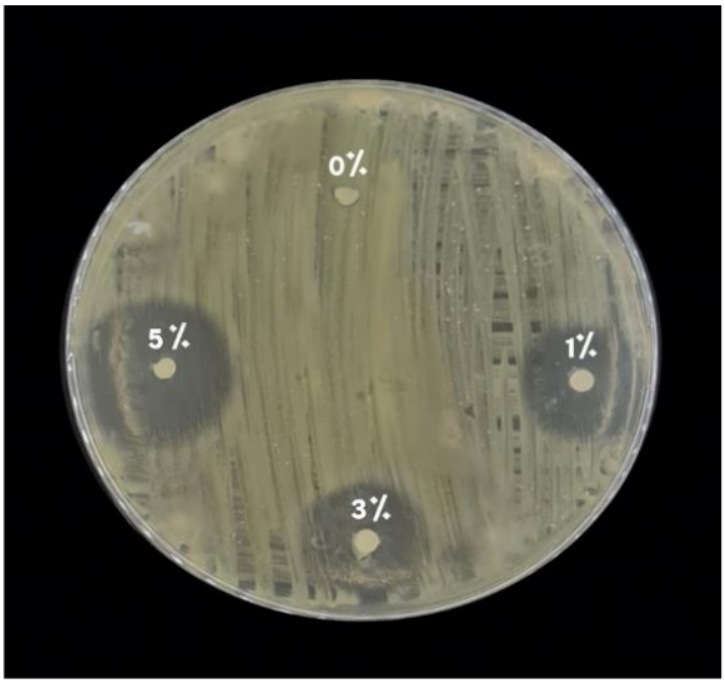

Figure (2) Antibacterial activity of (Tested materials) against *S. mutans*

List of tables:

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

Variable	Statistic	*P*-Value*
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): The descriptive statistics for the SBS of bonded groups

Variable	Mean	SD	Minimum	Maximum
Control	8.473	4.802	2.310	15.545
Modified with 1% SP oil	5.711	3.430	1.148	10.755
Modified with 3% SP oil	6.562	2.858	2.444	11.317
Modified with 5% SP oil	5.697	2.310	2.762	9.850

SD is the Standard Deviation, SP is the Salvadora persica essential oil, SBS measurement unit is MPa.

Table (3): One way analysis of variance for SBS among the groups.

	Sum of Squares	DF	Mean Square	F	*p*-value
Between Groups	47.574	3	15.858	1.347	0.275*
Within Groups	412.019	35	11.771		
Total	459.594	38			

DF is the degree of freedom, F ratio= MSB/MSE, *Not significant at *P≥0.05*

Table (4) descriptive statistics of adhesive remnant index (A.R.I)

Sample groups	Minimum	Maximum	Mean	Standard Deviation
Control 0%	1.00	4.00	2.50	1.732
Modified with 1% SP* oil	0.00	6.00	2.50	2.645
Modified with 3% SP oil	0.00	5.00	2.50	2.380
Modified with 5% SP oil	1.00	4.00	2.50	1.29099

*SP represents Salvadora persica.

Table (5) Kruskal-Wallis test of adhesive remnant index (A.R.I)

Kruskal-Wallis H	**0.069**
Degree of freedom	3
Asymp. Sig.	0.995

*$P \geq 0.05$

Table (6) Analysis of variance (ANOVA) for Antibacterial tests

	Sum of Squares	df	Mean Square	*P*-value
Between Groups	543.729	3	181.243	.001*
Within Groups	2.833	8	.354	
Total	546.563	11		

$P \leq 0.05$ there is a significant difference between groups.
DF is the degree of freedom.

Table (7) Antibacterial homogeneous Subsets Duncan test

Sample groups	1 mm	2mm	3mm	4mm
Control group	0.0000			
SP oil 1%		13.1667		
SP oil 3%			15.0000	
SP oil 5%				17.3333
Sig.	1.000	1.000	1.000	1.000