Article

Antibacterial Effect of Combinations of *Salvia officinalis* and *Glycyrrhiza glabra* Hydroalcoholic Extracts against *Enterococcus* spp.

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Abstract: *Enterococcus* spp. are a common culprit behind the failure of endodontic treatments, primarily due to their notorious resistance to antimicrobial agents. Considering this challenge, this study was conducted to assess the antimicrobial efficacy of a unique blend of hydroalcoholic extracts sourced from *Salvia officinalis* and *Glycyrrhiza glabra* against biofilms formed by *Enterococcus faecalis* and *Enterococcus faecium*. The chemical composition of these plant extracts was rigorously characterized, with primary compound quantification achieved through high-performance liquid chromatography (HPLC-DAD) analysis. Additionally, this study determined the minimal bactericidal concentrations of these extracts and evaluated their potential to combat biofilms by quantifying colony-forming units per milliliter (CFU/mL). The findings reveal that the simultaneous application of both extracts yielded additive and synergistic effects against *E. faecalis* and *E. faecium*, including both ATCC and clinical strains. Impressively, after a 24 h exposure, these extract combinations demonstrated efficacy comparable to that of a 0.12% chlorhexidine solution, establishing a statistically significant difference from the negative control group. Consequently, the concurrent use of these extracts emerges as a promising alternative antimicrobial strategy for addressing *Enterococcus* spp. in endodontic treatments, holding substantial potential for clinical applications in this context.

Keywords: anti-bacterial agents; *Salvia officinalis*; *Glycyrrhiza glabra*; *Enterococcus faecalis*; *Enterococcus faecium*

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1. Introduction

Endodontic infections are categorized as primary, secondary and persistent. A primary infection is a sequence of irreversible pulpitis that leads to dental pulp necrosis and periapical periodontitis [1]. Root canal treatment is generally successful in primary infection control [2]; however, in some cases, the treatment fails because of further contamination during or after the procedure, secondarily to professional intervention, resulting in a secondary infection, or because of the presence of resistant microorganisms that survived during the first treatment, resulting in persistent infection. In addition, the oral cavity may be subjected to other infections like periodontitis, peri-implantitis and others, which are caused by several microorganisms [3,4].

Enterococcus faecalis is a facultative anaerobic Gram-positive bacterium found in post-treatment periodontitis (secondary/persistent infections) [5] with a prevalence value of about 90% [6]. It is capable of escaping disinfection and has a resistance against antimicrobial agents [6] as it survives in an alkaline environment, tolerates a lack of nutrients, and deeply invades the dentinal tubules [7]. Furthermore, this microorganism was detected in subgingival biofilms of patients suffering from periodontitis and gingivitis [8].

In addition, *Enterococcus faecium* is another Gram-positive bacterium. It is found in the root canal of patients with post-treatment apical periodontitis [5,9], and it is found in the plaque of patients with poor oral hygiene and a history of smoking, playing a role in development of periodontal diseases [10]. These findings underscore the role of *E. faecium* in oral health issues and its potential impact on both endodontic and periodontal conditions.

Phytotherapy is gaining increasing attention in dentistry [11–13], particularly in specialized areas such as endodontics [14,15], or as a coating material [16,17] because of its antimicrobial action and biocompatibility. Its use reflects a broader trend towards exploring botanical solutions for dental applications, capitalizing on their well-established antimicrobial qualities and compatibility with oral health treatments. *Salvia officinalis*, popularly known as sage, is a Mediterranean plant belongs to Lamiaceae family, commonly used as a condiment. It has a broad antimicrobial action against Gram-positive and Gram-negative bacteria including *E. faecalis* [18–20], and antifungal action against *Candida albicans* [21]. In addition, *Glycyrrhiza glabra*, popularly known as liquorice, is a plant native to Western Asia, North Africa, and Southern Europe, belonging to the Fabaceae family, and has antimicrobial and antifungal action [22,23].

As far as we know, there are no studies in the literature that have evaluated the antimicrobial action of the combination of *Salvia officinalis* and *Glycyrrhiza glabra* against *Enterococcus* spp. Therefore, this study aimed to assess the antimicrobial action of combinations of *Salvia officinalis* and *Glycyrrhiza glabra* hydroalcoholic extracts against *Enterococcus faecalis* and *Enterococcus faecium* biofilms. The null hypothesis was that the combinations of *Salvia officinalis* and *Glycyrrhiza glabra* hydroalcoholic extracts exert no antimicrobial action against *Enterococcus* spp.

2. Materials and Methods

2.1. Plants Extracts

Two commercially acquired extracts from *S. officinalis* and *G. glabra* (Apis Flora®, Ribeirão Preto, SP, Brazil) were utilized in this study, accompanied by detailed reports and specifications provided by the manufacturer. These extracts were carefully prepared at a concentration of 3.5%, equivalent to 35 mg of extract powder, and were then diluted in 1 mL of hydroethanolic alcohol. It is important to note that the utilization of plant components in this research adhered to international, national, and institutional guidelines to ensure ethical and responsible research practices.

2.2. High-Performance Liquid Chromatography (HPLC) Analyses

The composition of markers in natural extracts was characterized using high-performance liquid chromatography (HPLC). For this analysis, an advanced liquid chromatograph with diode-array detection (HPLC-DAD) and an automated injector, the Merck-Hitachi D-7000 model (Merck KGaA, Darmstadt, Germany), were employed.

The chromatographic conditions employed consisted of a mobile phase composed of two solvents: solvent A, a mixture of water and formic acid (PA, Merck) at a ratio of 95:5, and solvent B, high-performance liquid chromatography (HPLC)-grade methanol sourced from Merck (Merck KGaA, Darmstadt, Germany). The flow rate was consistently maintained at 1 mL/min, and a linear gradient was applied, commencing at 0% B and gradually increasing to 70% B over a span of 50 min. Detection was performed at two specific wavelengths, namely 280 and 340 nm [24].

2.3. Inoculum Preparation

In this study, a reference strain sourced from the American Type Culture Collection (ATCC) (Rio de Janeiro, RJ, Brazil) was employed. Specifically, the reference strains included *E. faecalis* ATCC 4083 and *E. faecium* ATCC 6569. In addition to these reference strains, three clinical strains of *E. faecalis* and two clinical strains of *E. faecium* were used for the experiments. These clinical strains were supplied by the National Institute of Quality Control in Health (INCQS) of the Oswaldo Cruz Foundation (FIOCRUZ), as outlined in

Table 1. This comprehensive selection of strains allowed for a robust assessment of the antimicrobial properties of the *Salvia officinalis* and *Glycyrrhiza glabra* hydroalcoholic extracts against *Enterococcus* spp., encompassing both reference and clinically relevant strains.

Table 1. Antibiotic sensitivity classification for clinical isolates of *E. faecalis* and *E. faecium* obtained from root canals.

Bacteria	Strain	AC	XL	EM	AZ	TC	CI	VA
E. faecalis	1	S	S	S	S	S	S	S
	2	S	S	I	S	R	I	I
	3	S	S	S	S	R	S	I
E. faecium	1	S	S	I	R	S	S	S
	2	S	S	S	S	S	S	S

Legend: S = sensitive, I = intermediate, R = resistant, AC = amoxicillin, XL = amoxicillin + clavulanic acid, EM = erythromycin, AZ = azithromycin, TC = tetracycline, CI = ciprofloxacin, VA = vancomycin.

Before conducting experiments, all bacterial strains were cultured on Mueller–Hinton agar (HiMedia®, Mumbai, India) and incubated at 37 °C for a duration of 24 h to ensure optimal growth. A standardized bacterial inoculum was subsequently prepared for each strain and adjusted with precision using a spectrophotometer (Micronal B-582, Micronal, São Paulo, SP, Brazil) set at a wavelength of 530 nm, yielding an optical density of 0.284. This calibration resulted in a bacterial concentration of 1×10^6 cells per milliliter, providing a consistent and reproducible starting point for the subsequent experiments.

2.4. Minimum Inhibitory (MIC) and Minimum Bactericidal (MBC) Concentrations

In this study, the microdilution method, in accordance with the guidelines set forth by the Clinical and Laboratory Standards Institute (CLSI) documents M7-A9, was employed to determine the MIC and MBC values of hydroalcoholic extracts derived from *S. officinalis* and *G. glabra* when tested against *E. faecalis* and *E. faecium*.

For each bacterial strain, separate 96-well microplates (TPP, Trasadingen, Switzerland) were utilized. Initially, 100 µL of Mueller–Hinton broth (HiMedia®, Mumbai, India) was added to each well. Subsequently, 100 µL of the respective hydroalcoholic extract was introduced into the first well, wherein ten serial dilutions were meticulously performed. Following this, 100 µL of the corresponding bacterial inoculum was introduced into each well. These microplates were then placed in an incubator and maintained at 37 °C for a duration of 48 h. The MIC for each hydroalcoholic extract was identified in the last well of the microplate that exhibited no turbidity, indicating the absence of microbial growth.

To ascertain the MBC of the extracts, 100 µL of the MIC, as well as 100 µL of concentrations both above and below the MIC, were inoculated onto Brain Heart Infusion agar (Kasvi, São José dos Pinhais, PR, Brazil). After a 24 h incubation period, the MBC for each extract against each bacterial strain, encompassing clinical and standard strains, was determined by identifying plates where no colony growth was observed. Control groups were included, with a 0.12% chlorhexidine digluconate solution and sterilized saline solution (0.85% NaCl) serving as reference points for comparison. This meticulous methodology allowed for a comprehensive assessment of the antimicrobial potential of *S. officinalis* and *G. glabra* hydroalcoholic extracts against these bacterial strains.

2.5. Combined Extracts' Synergistic Effects

The checkerboard method was employed, as per the approach outlined by an anterior study [25], with certain modifications. This technique draws inspiration from the microdilution method of the Clinical and Laboratory Standards Institute. A serial dilution of the initial hydroalcoholic extract (50 µL/well) was carried out along the x-axis (horizontal) of the microplate. Likewise, another dilution of the second hydroalcoholic extract occurred along the y-axis (vertical) of the microplate, thus associating diverse extract concentrations.

Following this, 50 μL/well of BHI broth was introduced. Subsequently, a standardized inoculum (1×10^6 cells/mL) of each strain, amounting to 100 μL, was added, resulting in a total of 200 μL/well. The control group encompassed the culture medium without any extracts or inoculums, serving as a reference for comparison throughout the experiment. This method allowed for the evaluation of antimicrobial activity and interaction between different extract concentrations.

The microplates were incubated at a temperature of 37 °C for a duration of 48 h to facilitate subsequent visual examination. To evaluate the cooperative effect of the hydroalcoholic extracts, we utilized the fractional inhibitory concentration (FIC) index. This index classifies combinations into four categories: synergistic, additive, indifferent, or antagonistic. The calculation of the FIC index was based on the following formula: the FIC index, calculated as the sum of FIC1st and FIC2nd, embodies the relationship between two extracts. It is determined by dividing the minimum bactericidal concentration (MBC) of the first extract when used in combination by its MBC when used alone, and likewise for the second extract. Synergy was characterized when the FIC was equal to or less than 0.5, additivity when falling within the range of greater than 0.5 to less than or equal to 1.0, indifference when the FIC exceeded 1 but remained less than or equal to 4, and antagonism when the FIC exceeded 4.0, according to Moreno et al. [22].

2.6. Antibiofilm Activity

Fresh inocula were standardized to a concentration of 10^7 cells/mL, following the procedure detailed above. Subsequently, 200 μL/well of the bacterial inoculum was introduced into the microplates and allowed to incubate for a duration of 90 min at 37 °C. This period facilitated the initial attachment of bacterial cells to the well surfaces. Following this, the supernatant was carefully removed, making way for the addition of BHI broth. The subsequent incubation spanned 48 h to facilitate the formation of biofilms, and the culture medium was replaced after the initial 24 h of incubation.

After biofilm formation, two combinations were tested. Combination 1 was composed of 8.7 mg/mL of each extract, and combination 2 was composed of 4.3 mg/mL of each extract. Both combinations were applied for 5 min and 24 h. Chlorhexidine digluconate solution 0.12% and sterilized saline solution (0.85% NaCl) were used as control groups. Each experimental group consisted of n = 10. After exposure, the wells were washed with saline solution (200 μL/well) to remove cells affected by the extracts. The biofilms were disaggregated by an ultrasonic homogenizer (Sonic Vibra-cell—Biovera, Rio de Janeiro, RJ, Brazil) for 30 s and 25% power. The generated suspension was diluted and 5 μL of each dilution was added to BHI agar and incubated at 37 °C for 24 h. Colonies were counted in each drop and mean values were presented in CFU/mL (Log_{10}).

2.7. Statistical Analysis

In this study, all data were analyzed using GraphPad Prism 5.0 (San Diego, CA, USA), and for homogeneity, the Bioestat 5.0 software was used. Firstly, to analyze the data, three normality tests were used, including Shapiro–Wilk, Kolmogorov–Smirnov and the D'Agostino and Pearson omnibus. Later, normally distributed data were analyzed via ANOVA and Tukey's test, and non-normally distributed data were analyzed using the Kruskal–Wallis test and Dunn's test. The significance level of all tests was 5%.

3. Results

3.1. High-Performance Liquid Chromatography (HPLC) Analysis

3.1.1. Salvia Officinalis Extract

Through chromatographic analysis, it was observed that some chlorogenic acid derivatives were found at 11.36 min, rutin was found at 27.09 min and some flavonoids which could be apigenin were found at 30.69 min (Figure 1).

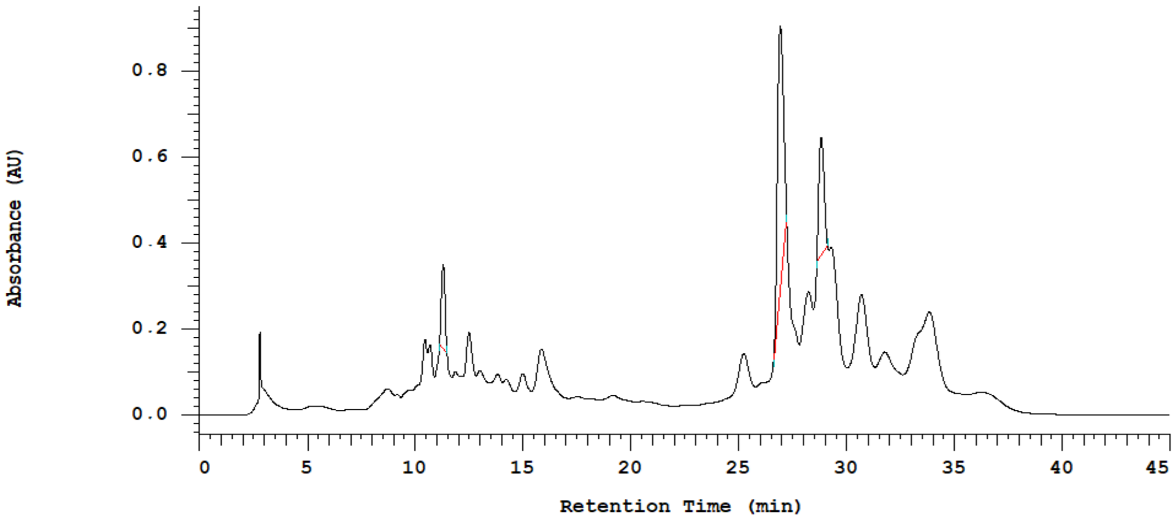

Figure 1. Structures of identified compounds isolated from the *Salvia officinalis* extract: chlorogenic acid, flavonoids (apigenin) and rutin.

3.1.2. Glycyrrhiza Glabra Extract

Through chromatographic analysis, some liquiritigenin and isoliquiritigenin derivatives were observed at the retention time (Rt) of 17.78 and 20.81 min (Figure 2).

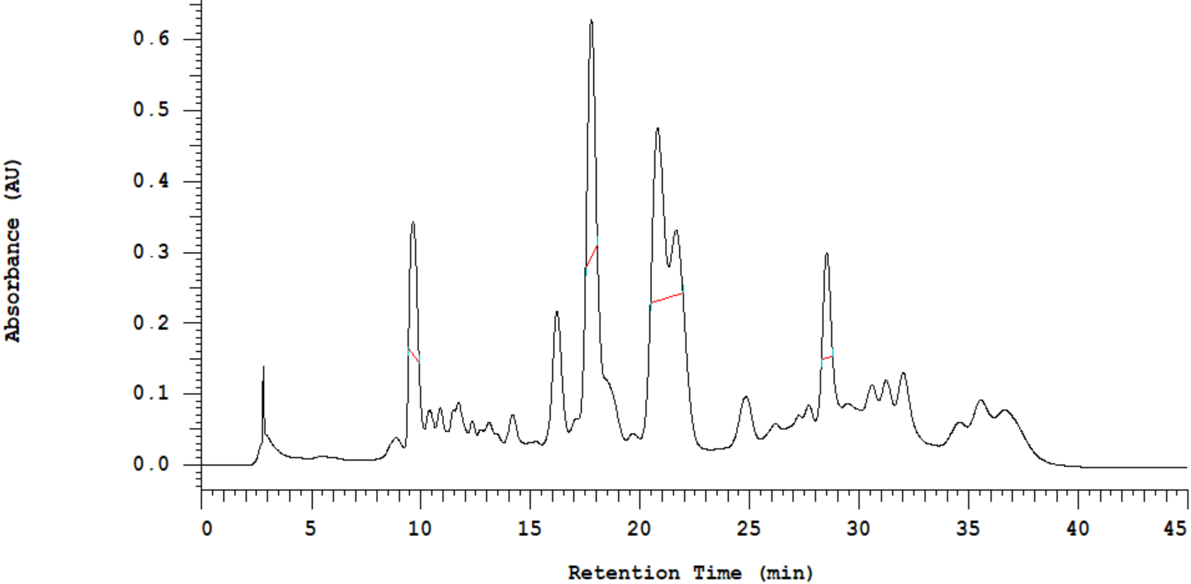

Figure 2. Structures of identified compounds isolated from the *Glycyrrhiza glabra* extract: liquiritigenin and isoliquiritigenin.

3.2. Minimum Inhibitory (MIC) and Minimum Bactericidal (MBC) Concentrations

In this study, determining the minimum inhibitory concentration (MIC) values was rendered impractical due to the coloration of the extracts, which made visual assessment unfeasible. However, the minimum bactericidal concentration (MBC) values for the *S. officinalis* and *G. glabra* hydroalcoholic extracts were successfully determined and are detailed in Table 2. These MBC values ranged from 1.0 to 7.7, providing essential information about the antimicrobial effectiveness of the extracts against the tested microorganisms.

Table 2. Minimum bactericidal concentration (MBC) values of *S. officinalis* and *G. glabra* hydroalcoholic extracts.

Bacterial Strain	*S. officinalis* MBC (mg/mL)	*G. glabra* MBC (mg/mL)
E. faecalis ATCC	4.3	4.3
E. faecalis clinical strain 1	2.1	4.3
E. faecalis clinical strain 2	1.0	4.3
E. faecalis clinical strain 3	8.7	4.3
E. faecium ATCC	8.7	8.7
E. faecium clinical strain 1	2.1	2.1
E. faecium clinical strain 2	4.3	4.3

3.3. Combined Extracts' Synergistic Effects

The combination of hydroalcoholic extracts derived from *S. officinalis* and *G. glabra* exhibited both additive and synergistic effects when assessed using the fractional inhibitory concentration (FIC) index (Table 3). Specifically, these effects were observed against *E. faecalis* ATCC, clinical strain 1 of *E. faecalis, E. faecium* ATCC, and clinical strain 1 of *E. faecium*. It is worth noting, however, that the results were either indifferent or demonstrated antagonist effects when tested against the remaining clinical strains.

Table 3. Minimum bactericidal concentration (MBC) values in mg/mL, in addition to the combined effect of *S. officinalis* and *G. glabra* hydroalcoholic extracts, against *Enterococcus* spp.

Bacterial Strain	Isolated Extract MBC Value (mg/mL)		Combined Concentrations (mg/mL)		FIC Index	Reduction in MIC		Effect
	S. officinalis	*G. glabra*	*S. officinalis*	*G. glabra*		*S. officinalis*	*G. glabra*	
E. faecalis ATCC	4.3	4.3	2.1	2.1	0.97	2	2	Add
			2.1	1.0	0.72	2	4	Add
			2.1	0.5	0.60	2	8	Add
			2.1	0.2	0.53	2	21	Add
			2.1	0.1	0.51	2	43	Add
			2.1	0.06	0.50	2	71	Syn
			0.5	2.1	0.6	8	2	Add
			0.2	2.1	0.53	21	2	Add
			0.1	2.1	0.51	43	2	Add
			0.06	2.1	0.50	71	2	Syn
			0.03	2.1	0.49	143	2	Syn
			0.01	2.1	0.49	430	2	Syn
			0.007	2.1	0.49	614	2	Syn
E. faecalis Clinical strain 1	2.1	4.3	1.0	1.0	0.70	2	4	Add
			1.0	0.5	0.59	2	8	Add
			1.0	0.2	0.52	2	21	Add
			1.0	0.1	0.49	2	43	Syn
			1.0	0.06	0.49	2	71	Syn
E. faecium ATCC	8.7	8.7	4.3	4.3	0.98	2	2	Add
			4.3	2.1	0.73	2	4	Add
			4.3	1.0	0.60	2	8	Add
E. faecium clinical strain 1	4.3	4.3	2.1	2.1	0.97	2	2	Add
			1.0	1.0	2.1	0.72	4	Add

Legend: Add: Additive effect. FIC index = FIC1st + FIC2nd = (MBC of the 1st extract in combination/MBC of the 1st extract alone) + (MBC of the 2nd extract in combination/MBC of the 2nd extract alone). The results were interpreted as a synergistic (≤0.5), additive (>0.5 and ≤1.0), indifferent (>1.0 and <4.0) or antagonist effect (≥4.0) [25].

3.4. Combined Extracts' Antibiofilm Effect

Both Combination 1 and Combination 2 displayed impressive effectiveness in reducing *E. faecalis* biofilms, including both ATCC and clinical strains. These combinations exhibited statistically significant differences when compared to the control group. Notably, both Combination 1 and Combination 2 were equally as effective as the chlorhexidine group, both after a 5 min exposure and a 24 h exposure. However, it is important to highlight that while Combination 2 yielded positive results against most strains, it was not effective against *E. faecalis* clinical strains 1 and 3 after a 5 min exposure (as indicated in Figure 3). This suggests some variability in their response to the treatment, emphasizing the importance of considering specific strain characteristics when applying these combinations in a clinical context.

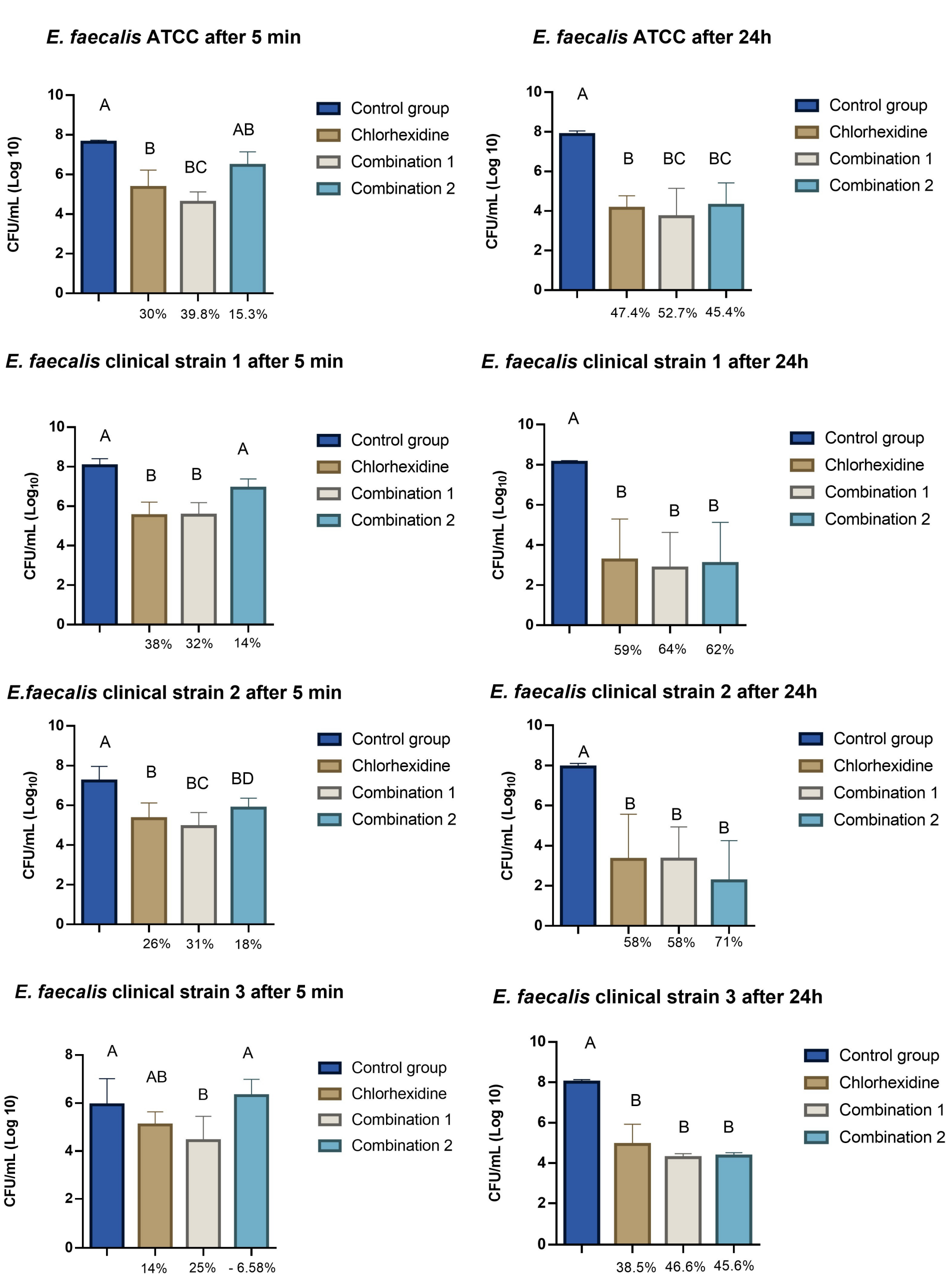

Figure 3. The reduction in *E. faecalis* biofilm load following treatment with combinations of *S. officinalis* and *G. glabra* hydroalcoholic extracts, administered for both 5 min and 24 h, was evaluated. In the legend, distinctions are made using different uppercase letters to signify statistically significant differences between the various treatment groups.

Both Combination 1 and Combination 2 demonstrated remarkable efficacy in reducing *E. faecium* biofilms, encompassing both ATCC and clinical strains. These combinations exhibited statistically significant differences when compared to the control group. Furthermore, both Combination 1 and Combination 2 proved to be equally as effective as the chlorhexidine group, both following a 5 min exposure and a 24 h exposure. However, it is worth noting that Combination 2, while generally effective, did not show efficacy against *E. faecium* clinical strain 2 and strain 3 after a 5 min exposure, as indicated in Figure 4. This outcome suggests that the response to treatment may vary among different strains, underscoring the need to consider strain-specific characteristics when applying these combinations in clinical settings.

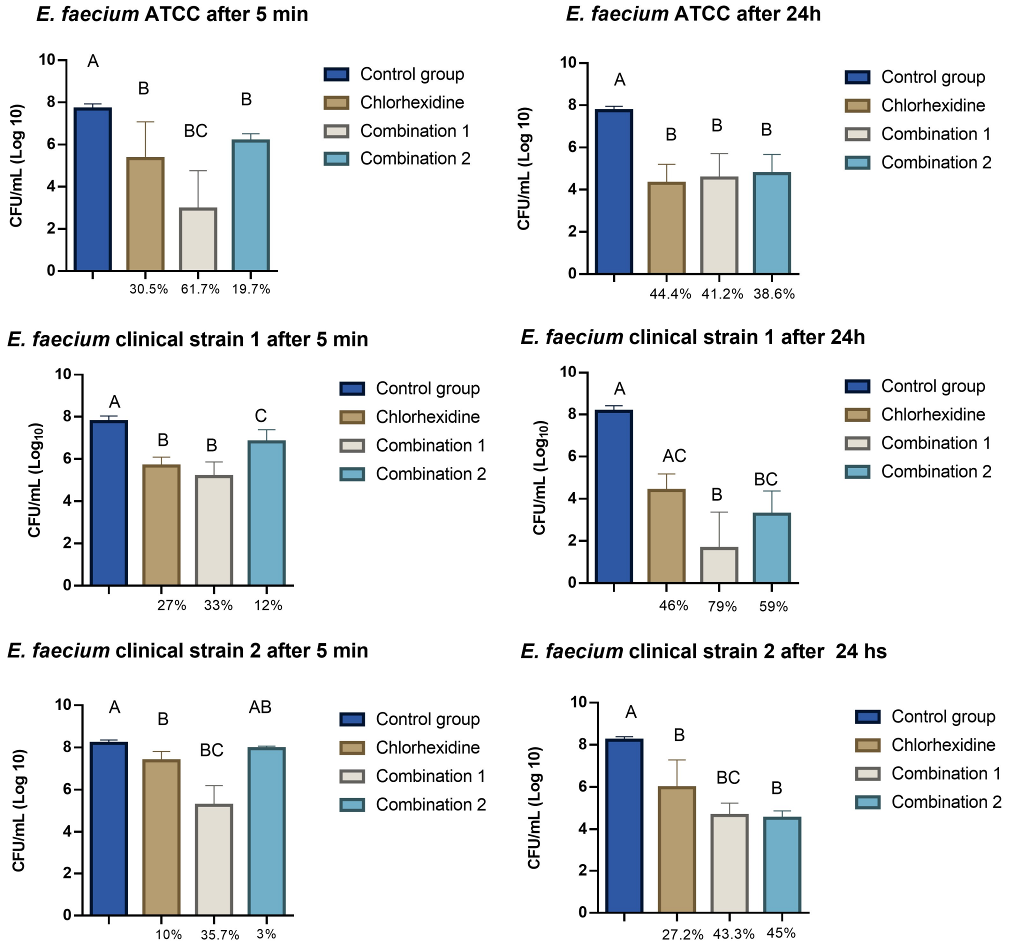

Figure 4. The reduction in *E. faevium* biofilm load following treatment with combinations of *S. officinalis* and *G. glabra* hydroalcoholic extracts, administered for both 5 min and 24 h, was evaluated. In the legend, distinctions are made using different uppercase letters to signify statistically significant differences between the various treatment groups.

4. Discussion

The resistance of *Enterococcus* spp. against a wide array of antimicrobial agents, as well as its ability to thrive in alkaline environments, tolerate nutrient deprivation, and penetrate deep into dentinal tubules, as highlighted in previous studies [7], underscores the pressing need for novel products exhibiting potent antibacterial properties and minimal toxicity, a goal pursued by numerous research endeavors [14,15].

This study unveiled the effectiveness of combinations comprising hydroalcoholic extracts from *S. officinalis* and *G. glabra* in diminishing the presence of *Enterococcus* spp. within both 5 min and 24 h. Consequently, the initial null hypothesis posited for this study was decisively rejected. These findings offer promising prospects for the development

of alternative antimicrobial approaches with the potential to combat *Enterococcus* spp., addressing a critical need in the field of dentistry and oral health.

In the present study, the identified compounds in *S. officinalis* hydroalcoholic extract were chlorogenic acid at 11.36 min, some flavonoids (apigenin) at 30.69 min and rutin at 27.09 min. In the literature, it was reported that *S. officinalis* extract is rich in flavonoids, rosmarinic acid, rutin, chlorogenic acid, and quercetin [26].

Conversely, both liquiritigenin and isoliquiritigenin derivatives were abundantly found at 17.78 min and 20.81 min in *G. glabra* hydroalcoholic extract. Liquiritigenin and isoliquiritigenin are the main flavonoids of licorice extract, with intense biological activity reported [27]. Our results are similar to those of other studies in the literature, which confirmed the presence of these flavonoids in *G. glabra* [28,29].

The combined application of hydroalcoholic extracts from *S. officinalis* and *G. glabra* yielded promising results, demonstrating both additive and synergistic effects against various strains of *Enterococcus*. Specifically, these effects were observed in *E. faecalis* ATCC, *E. faecalis* clinical strain 1, *E. faecium* ATCC, and *E. faecium* clinical strain 1. Two distinct combinations, Combination 1 (comprising 8.7 mg/mL of each extract) and Combination 2 (comprising 4.3 mg/mL of each extract), emerged as particularly effective. These combinations significantly reduced *E. faecalis* and *E. faecium* biofilms, including both ATCC and clinical strains, after a 24 h exposure period. Impressively, their efficacy was on par with that of a 0.12% chlorhexidine solution, and these combinations exhibited a statistically significant difference when compared to the control group. These findings underscore the potential of these extract combinations as a promising alternative for combating *Enterococcus* biofilms, representing a significant advancement in the pursuit of effective antimicrobial strategies for oral health applications.

As far as we are aware, this is the first study to evaluate the antibiofilm action of combinations of *S. officinalis* and *G. glabra* hydroalcoholic extracts against *Enterococcus* spp., making this study a pioneer. However, in the literature, *S. officinalis* extract was evaluated for its use as an endodontic irrigant, showing that it was effective against *E. faecalis* biofilms in infected root canals [30]. In addition, in another study, *G. glabra* was evaluated as an antimicrobial agent in endodontic treatment, and it was found that the extract eliminated the planktonic cells of *E. faecalis*, but it was not able to eradicate the biofilms grown on dentin discs [31]. Furthermore, in another study, with the same finality, 25% *G. glabra* was effective as intracanal medication against *E. faecalis* [32]. While these prior studies focused on the individual efficacy of these extracts in specific contexts, our study takes a novel approach by assessing their combined antibiofilm activity against *Enterococcus* spp. This novel perspective adds valuable insights to the potential applications of these extracts in addressing oral health challenges.

The antibacterial properties of *G. glabra* extract can be attributed to its composition, notably the presence of liquiritigenin. In a murine lung infection model, *G. glabra* extract demonstrated therapeutic efficacy against a multidrug-resistant strain of *P. aeruginosa* [33]. In addition, it has been shown to have antifungal activity against *C. albicans* in different studies, because of its richness in liquiritigenin, liquiritin, licochalcone A and glabridin [34,35]. Furthermore, the study of Marcoux et al. (2020) [36] revealed that flavonoids derived from licorice possess antibacterial activity against *Enterococcus faecalis* biofilms. These flavonoids were found to reduce the metabolic activity of the biofilm by 28% and 29%, indicating their potential in combating biofilm-related infections. This body of research highlights the multifaceted antimicrobial properties of *G. glabra* extract and its constituent compounds, shedding light on their effectiveness against a range of pathogens, including multidrug-resistant strains and biofilm-forming bacteria.

The presence of chlorogenic acid in *S. officinalis* is noteworthy due to its potent biological activity, as documented in the literature. This phenolic compound has been shown to exhibit pronounced antibacterial effects against *E. coli*. It achieves this by disrupting the structural integrity of the bacterial cell wall and membrane. This disruptive action is attributed to the high polarity and strong affinity of chlorogenic acid for lipids. Conse-

quently, the compound binds effectively to the surface of Gram-negative bacteria, such as *E. coli*, and induces alterations in the membrane structure. This process leads to an increase in membrane permeability, resulting in the leakage of cellular contents. These findings shed light on the mechanism through which chlorogenic acid exerts its antibacterial action, offering valuable insights into its potential applications in combating bacterial infections, particularly those involving Gram-negative bacteria like *E. coli* [37].

In addition, rutin has been reported as a promising antibacterial and antifungal compound for use in the drug delivery system [38], and apigenin has low toxicity and is practically insoluble in highly polar solvents such as water. Flavonoids are also more soluble in methanol than in water [39]. With regard to their action against microorganisms, these compound have anti-caries actions, as they were able to inhibit the synthesis of water-insoluble glucans, which are produced by *S. mutans* for biofilm formation, and consequently reduce the incidence of dental caries with minimal effects on the oral microbiota in vivo [40]. These findings underscore the multifaceted potential of these compounds, both in terms of their antimicrobial properties and their suitability for various pharmaceutical and delivery applications.

Lastly, this study recommends the utilization of hydroalcoholic extracts from *Salvia officinalis* and *Glycyrrhiza glabra* for various applications in the realm of dentistry. These versatile extracts could be employed as pharmaceutical coatings, surface coatings, coatings for drug delivery vehicles, or as antimicrobial agents in endodontic treatments. Additionally, they hold promise as components in mouthwash or toothpaste formulations, thanks to their demonstrated antibiofilm properties against *Enterococcus* spp. These findings open up new avenues for harnessing the antimicrobial potential of these plant extracts to improve oral health and enhance the effectiveness of dental treatments.

5. Conclusions

The hydroalcoholic extracts of *S. officinalis* and *G. glabra*, when combined, exhibited both additive and synergistic effects against *E. faecalis* and *E. faecium*. The highest level of effectiveness was observed after a 24 h period, particularly against biofilms.

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