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Evaluation of Antibacterial Activity for Aqueous Exteracted Combination of Triphala and Aloe-Vera Against *Streptoccocus Oralis*: (In Vitro Study)

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Abstract

Background: *Streptoccocus Oralis* is an early colonizer in dental biofilm and contributes to plaque formation and periodontal inflammation. The search for safe, natural antimicrobial alternatives has increased interest in herbal agents such as Triphala and Aloe vera. However, their combined activity against S. oralis has not been fully assessed alongside phytochemical profiling.

Aim: To evaluate the antibacterial activity of aqueous-extracted Triphala, Aloe vera, and their combination against *S. oralis*, including inhibition zone measurement, MIC, MBC, and HPLC characterization.

Materials and Methods: Aloe vera gel and Triphala powder were extracted using aqueous extraction and freeze-drying. Antibacterial activity was assessed using agar well-diffusion, MIC, and MBC assays at concentrations of 20–100%. Chlorhexidine 0.12% served as the positive control. Statistical analysis included one-way ANOVA and LSD tests. Phytochemical profiling of Triphala was performed using HPLC.

Results: The combination extract demonstrated the strongest antibacterial effect, producing inhibition zones of 18.75–25.25 mm, significantly higher than Aloe vera, Triphala alone, and chlorhexidine 0.12% (mean CHX = 13–14 mm; $p < 0.05$).

The MIC of the combination extract was 5–10%, while the MBC was 20%, indicating potent inhibitory and bactericidal activity at low concentrations.

HPLC profiling of Triphala revealed abundant polyphenols including chebulagic acid (RT 3.51 min), chebulinic acid (RT 3.90 min), gallic acid (RT 4.33 min), ellagic acid (RT 4.92 min), rutin (RT 4.66 min), quercetin (RT 10.05 min), apigenin (RT 16.72 min), chlorogenic acid (RT 18.19 min), and catechin (RT 21.26 min). Vitamin analysis also identified a dominant Vitamin C peak at RT 4.89 min, supporting strong antioxidant potential.

HPLC of Aloe vera identified major anthraquinones including Aloin A/B (RT 5.03 min; 65% of total area), Aloe-emodin (RT 6.27 min), and Emodin (RT 10.14 min) compounds widely recognized for antibacterial, anti-biofilm, and membrane-disruptive effects.

The combined presence of Triphala polyphenols and Aloe vera anthraquinones explains the observed synergistic antibacterial effect against *S. oralis*.

Conclusion: The aqueous combination of Triphala and Aloe vera exhibits a synergistic, potent antibacterial effect against S. oralis, with inhibition zones surpassing chlorhexidine and demonstrating low MIC (5–10%) and bactericidal MBC (20%). Supported by rich polyphenolic and anthraquinone content identified by HPLC, this combination represents a promising natural alternative or adjunct to chemical antimicrobials for early plaque control.

Keywords: Triphala, Aloe vera, *Streptococcus oralis*, MIC, MBC, Chlorhexidine, HPLC, Aloin, Aloe-emodin, Polyphenols, Anthraquinones, Synergistic antibacterial activity, In-vitro study

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1. Introduction

Dental biofilm represents a structurally and functionally complex community of microorganisms that adheres to the tooth surface and becomes embedded within a matrix of salivary and bacterial polymers.[1,2] More than 700 bacterial species inhabit the oral cavity, and many of these species can readily adhere to the developing biofilm through the acquired pellicle—a glycoprotein-rich layer formed from salivary components on clean tooth surfaces.[3] Among the earliest colonizers of dental biofilm is *Streptococcus oralis (S. oralis)*, a pioneer Gram-positive coccus that attaches firmly to the salivary pellicle and provides binding sites for secondary colonizers, facilitating the maturation of the dental biofilm ecosystem.[4,5]

The accumulation and maturation of dental biofilm have long been recognized as major etiological factors in dental caries, gingivitis, and periodontal diseases.[6] Gingival inflammation originates from the metabolic and structural by-products of microbial biofilm at or near the gingival sulcus,[7] and persistent accumulation of pathogenic plaque is considered the principal risk factor for the progression to periodontitis.[8] Although mechanical plaque control using toothbrushes and interdental aids remains the cornerstone of oral hygiene, several studies have shown that mechanical methods alone often fail to completely remove biofilm deposits, especially in interproximal, subgingival, orthodontic, or anatomically complex sites that require high levels of dexterity and compliance.[9,10] Additionally, elderly individuals and patients with physical limitations frequently experience difficulties maintaining optimal plaque control.[11]

Because of these limitations, adjunctive chemotherapeutic agents have been widely recommended to enhance plaque control and reduce gingival inflammation. Chlorhexidine (CHX) mouthwash remains the most commonly prescribed chemical agent due to its broad-spectrum antimicrobial activity.[12] However, its use is associated with several drawbacks, including tooth and restoration staining, altered taste perception, oral mucosal irritation, and increased supragingival calculus formation.[13] These side effects limit patient compliance and necessitate the search for alternative antimicrobial agents that are effective, safe, biocompatible, and economically accessible.[13]

In recent years, herbal preparations have gained considerable attention as potential substitutes to conventional chemical antimicrobials. Medicinal plants and herbal extracts have been used for centuries and are increasingly preferred due to their natural origin, lower toxicity, and broad pharmacological properties, including antioxidant, anti-inflammatory, and antimicrobial effects.[14] Several herbal formulations have demonstrated significant anti-biofilm potential and have been proposed as safer alternatives to CHX in daily oral hygiene regimens.[15]

Among the promising herbal agents are Triphala and Aloe vera. Triphala, a classical Ayurvedic polyherbal formulation composed of Terminalia chebula, Terminalia bellirica, and Emblica officinalis, is rich in polyphenols such as gallic acid, ellagic acid, chebulinic acid, and quercetin, all of which are known for their potent antimicrobial and antioxidant effects.[16] Aloe vera contains anthraquinones including aloin A/B, aloe-emodin, and emodin, compounds with demonstrated antibacterial, anti-inflammatory, and wound-healing activities.[17] Both extracts have shown inhibitory effects against various oral pathogens, yet their combined antibacterial efficacy against S. oralis has not been thoroughly investigated.

Given the increasing demand for natural, safe, and cost-effective antimicrobial agents for plaque control—and considering the synergistic potential of combining polyphenol-rich Triphala with anthraquinone-rich Aloe vera—the present study was designed to evaluate the antibacterial activity of aqueous-extracted Triphala, Aloe vera, and their combination against Streptococcus oralis using inhibition zone measurement, MIC, MBC, and phytochemical characterization by HPLC.

2. Materials and methods

2.1. Ethical approval and study setting

The study procedure was approved by the Medical Ethical Committee, College of Dentistry, University of Baghdad. This in-vitro investigation was conducted at the Laboratory Unit of Altakadm Learning Center in Baghdad, Iraq.

2.2. Sample collection and dental biofilm sampling

Samples were collected from forty systemically healthy individuals (males and females) attending a private dental clinic with plaque-induced gingivitis. Supragingival plaque was obtained using a sterilized Gracey curette after isolating the tooth with cotton rolls and drying with gentle air spray to prevent contamination with saliva or gingival crevicular fluid.[18] The collected plaque was immediately transferred into 3 mL of Brain Heart Infusion Broth (BHI-B) in sterile tubes was transported to the microbiology laboratory for further processing.[19] Exclusion criteria included patients who had received antibiotics or antimicrobial mouth rinses within at least one month before the study

2.3. Isolation and identification of streptococcus oralis

2.3.1. The conventional method

This procedure was done for each plaque sample: Plaque samples were inoculated into Brain Heart Infusion Broth (BHI-B) and incubated for 4 hours at 37 °C in a 5% CO_2 environment then Bacteria from BHI-B were then sub-cultured onto Mitis Salivarius Agar and Blood Agar plates, which had been previously prepared, and incubated at 37 °C for 48 hours in a CO_2 incubator. Finally the pure colonies were further identified by Gram's staining and biochemical tests to confirm *Streptococcus oralis*.[20]

2.3.2. Molecular identification by polymerase chain reaction (PCR)

Genomic DNA was extracted from purified S. oralis isolates using a commercial bacterial DNA extraction kit according to the manufacturer's instructions. Molecular confirmation was performed by PCR amplification of species-specific housekeeping genes using validated primer sets as in Table 1 PCR products were analyzed by agarose gel electrophoresis, and the presence of amplicons of expected size was used to confirm the identity of S. *oralis*.[21]

2.3.2.1. Primers

Primers of this study were designed according to Hoshino et al., (2004)[22] and manufactured by Macrogen company, (Korea). All of these primers were summarized in this table:

2.3.2.2. PCR amplification of gtfR and gtfP genes

Molecular identification of these genes were done by Uniplex-PCR procedure. where a specific primer (primer list Table 1). PCR amplification of DNA was performed by thermal cycler in final mixture volume of 25 μl (GoTaq®G2 Green Master Mix, Promega, USA). PCR mixtures and conditions of this assay was summarized in Tables 2 and 3.

2.3.2.3. Gel electrophoresis

Gel electrophoresis is applied to ensure the validity of DNA extraction PCR results. 2% agarose concentration for the quality of the extracted DNA stained with 500μl of a 10mg/ml stock solution per 100 ml green star dye with 100 volts for 36 minutes. The results were confirmed by using Gel Documentation system (DUALED Blue / White Transilluminator, Bioneer, Korea).

2.4. Preservation of bacterial isolates

Pure colonies were inoculated into 3 ml of Brain Heart Infusion Broth (BHI-B) containing 20% glycerol and incubated at 37 °C for 24 h. After incubation, the culture tubes were sealed with parafilm and stored at −20 °C for long-term preservation.[23]

Table 1. Primer list in the current study.

Gene	Oligo Name	5′ - Oligo Seq - 3′	PCR product (bp)	Reference
gtfR	gtfR -F	TCCCGGTCAGCAAACTCCAGCC	374	
	gtfR -R	GCAACCTTTGGATTTGCAAC		*Hoshino et al., (2004)*[22]
gtfP	gtfP -F	GGATAGTGGCTCAGGGCAGCCAGTT	313	
	gtfP -R	GAACAGTTGCTGGACTTGCTTGTC		Hoshino et al., (2004)[22]

Table 2. Uniplex PCR mixtures and conditions for identification of gtfR gene.

PCR mixtures		PCR conditions		
Contents	Volume	Type of cycle	Condition	No. of cycles
Master Mix	12.5 μl	Initialization	95 °C for 5 min	1
Forward Primer	2.5 μl	Denaturation	94 °C for 1 min	35
Reverse Primer	2.5 μl	Annealing	66 °C for 1 min	
Template DNA	3 μl	Extension	72 °C for 1 min	
Nuclase-Free Water	4.5 μl	Final Extension	72 °C for 10 min	1

Table 3. Uniplex PCR mixtures and conditions for identification of gtfP gene.

PCR mixtures		PCR conditions		
Contents	Volume	Type of cycle	Condition	No. of cycles
Master Mix	12.5 μl	Initialization	95 °C for 5 min	1
Forward Primer	2.5 μl	Denaturation	94 °C for 1 min	35
Reverse Primer	2.5 μl	Annealing	70 °C for 1 min	
Template DNA	3 μl	Extension	72 °C for 1 min	
Nuclase-Free Water	4.5 μl	Final Extension	72 °C for 10 min	1

2.5. Preparation of herbal extracts

2.5.1. Triphala extract

Triphala powder, consisting of equal parts of Terminalia chebula, Terminalia bellirica, and Emblica officinalis, was procured from a certified herbal supplier. Ten grams of the powder were extracted in 100 mL of distilled water by continuous stirring at 60 °C for 4 hours. The solution was filtered through Whatman No.1 filter paper, and the filtrate was concentrated using a freeze-dryer to obtain a dried aqueous extract. Both dried extracts were weighed, stored in airtight containers at 4 °C, and later reconstituted in sterile distilled water to prepare different working concentrations.[16,24]

2.5.2. Aloe vera extract

To obtain aqueous extracts of Aloe vera gel and Triphala powder, fresh Aloe vera leaves were collected from local farms, washed thoroughly with distilled water, and the outer rind was carefully removed under aseptic conditions to obtain the inner gel. The gel was homogenized using a sterile blender, diluted in distilled water (1:10 w/v), and centrifuged at 5000 rpm for 15 minutes to remove fibers. The supernatant was filtered through Whatman No.1 filter paper, and the filtrate was freeze-dried to obtain a dry powder, which was preserved in tightly sealed amber bottles at 4 °C until further use.[25]

2.5.3. Preparation of different concentrations

To prepare the different concentrations (conc.) for the aqueous Aloe vera and Triphala extracts, vortex mixer was used to obtain homogeneous solutions. Sterile distilled water acted as the solvent for diluting the extracts to various concentrations.[24,25] The identical method was employed for both extracts, detailed as follows.

1.1 g of the extract was dissolved in 5 mL of sterile distilled water to achieve a 20% concentration.

2.2 g of the extract was dissolved in 5 mL of sterile distilled water to achieve a 40% concentration.

3.3 g of the extract was dissolved in 5 mL of sterile distilled water to achieve a 60% concentration.

4.4 g of the extract was dissolved in 5 mL of sterile distilled water to achieve a 80% concentration.

5.5g of the extract was dissolved in 5 mL of sterile distilled water to achieve a 100% concentration.

2.5.4. Preparation of combination extract

According to the method demonstrated by Stephenson (2010), the mixing procedure for each concentration was carried out by combining equal volumes (1:1) of Aloe vera aqueous extract and Triphala aqueous extract of the same concentration. For example, 1 mL of 20% Aloe vera extract was combined with 1 mL of 20% Triphala extract to produce a 20% combination extract. The same procedure was followed for 40%, 60%, 80%, and 100% concentrations. A vortex mixer was used to obtain homogeneous solutions before use in antibacterial assays.[26]

2.6. Determination of the minimum inhibitory concentration (MIC) and MBC of extract

The minimum inhibitory concentration (MIC) of the aqueous combination of *Triphala* and *Aloe vera* extracts against *Streptococcus oralis* was determined using the broth microdilution method according to CLSI guidelines,[27,28] with minor modifications. Aqueous extracts of *Triphala* and *Aloe vera* were prepared separately and combined in equal volumes (1:1), using sterile distilled water as the extraction solvent. Two-fold serial dilutions of the combined extracts were prepared in sterile 96-well microtiter plates, generating concentrations ranging from 20% to 100%. Chlorhexidine 0.12% served as the positive control, whereas sterile distilled water was used as the negative control.[29] Standardized bacterial suspensions were prepared by adjusting overnight cultures of *S. oralis* to 0.5 McFarland (approximately 1×10^8 CFU/ml), followed by dilution to 1×10^6 CFU/ml in Mueller–Hinton broth supplemented with 5% sheep blood.[30,31] Each well received 100 μl of bacterial inoculum and 100 μl of extract dilution, yielding a final volume of 200 μl. The plates were incubated aerobically at 37 °C for 24 hours. The MIC was defined as the lowest concentration of the extract that exhibited no visible turbidity compared with the control well.[27,28] To confirm bactericidal activity, 10 μl from wells exhibiting no visible growth were subcultured onto Mueller–Hinton agar plates and incubated for an additional 24 hours, where the absence of colony formation identified the minimum bactericidal concentration (MBC).[27,32]

2.7. Evaluation of the antibacterial activity of extracts

The antibacterial activity of the aqueous combination of Triphala and Aloe vera extracts was evaluated using the agar well diffusion method.[27,33] Fresh aqueous extracts of both plants were prepared and filtered to ensure sterility, then combined at a 1:1 ratio. The mixture was subsequently diluted with sterile distilled water to obtain working concentrations of 20%, 40%, 60%, 80%, and 100%. Chlorhexidine 0.12%, prepared from a 2% stock solution using the dilution equation (C1V1 = C2V2), served as the positive control, whereas sterile distilled water was used as the negative control.[29] Standardized bacterial suspensions of *Streptococcus oralis* were prepared from

overnight cultures and adjusted to 0.5 McFarland turbidity (approximately 1×10^8 CFU/ml) before inoculating Mueller–Hinton agar plates. Wells of 6 mm diameter were aseptically punched into the agar, and 50 μl of each extract concentration or control solution was dispensed into the wells. The plates were incubated aerobically at 37 °C for 24 hours. Following incubation, the diameters of inhibition zones were measured in millimeters, and each experiment was conducted in quadruplicate, with mean values calculated to determine antibacterial efficacy.[27,34]

2.8. Determination of active constituents of triphala and aloe vera using high-performance liquid chromatography (HPLC)

The phytochemical composition of Triphala and Aloe vera aqueous extracts was determined using High-Performance Liquid Chromatography (HPLC) to identify and quantify the major bioactive constituents contributing to their antibacterial activity.

HPLC Analysis

HPLC analysis was carried out using a SYKAM,) Germany (system equipped with a quaternary pump, auto-sampler, and UV detector. Separation of the phytochemical compounds was achieved on a C18 reverse-phase column C18-ODS (25cm *4.6 mm). The mobile phase consisted of Solvent A formic acid (79 :25 :5) and Solvent B) methanol: D.W(, applied using a gradient elution program. The flow rate was maintained at 1 ml/min, and the injection volume was 20 μl. Detection was performed at 254 nm for phenolic acids and tannins, and between 280–360 nm for flavonoids Retention times and peak areas were compared with standard compounds including gallic acid, ellagic acid, quercetin, and catechin.[16,35]

2.9. Descriptive statistics

Descriptive statistics, including means and standard deviations (SD), were calculated and presented graphically. Inferential statistics were performed using one-way ANOVA and LSD compare differences among the extract concentrations, chlorhexidine 0.12%, and sterile distilled water and t-tests for bacterial strain (S. *oralis*).

3. Results

3.1. Isolation and identification of S. oralis

Based on the conventional identification methods—including culture characteristics on Mitis Salivarius agar bluish, convex, soft colonies with a slightly frosted or “gumdrop” appearance and blood agar)the colonies appeared small, round, translucent, and exhibited α-hemolysis, producing a subtle greenish discoloration around the colonies(, microscopic examination using Gram staining)revealed Gram-positive cocci arranged in chains, consistent with *viridans* group *streptococci* (and catalase test negative and optochin resistance as Fig. 1. The results demonstrated that out of the 30 examined samples, 5 (16.6%) were classified as **positive**, indicating the confirmed presence of *Streptococcus oralis*. In contrast, 25 samples (83.4%) were categorized as **negative**, meaning that *S. oralis* was not detected in these samples.

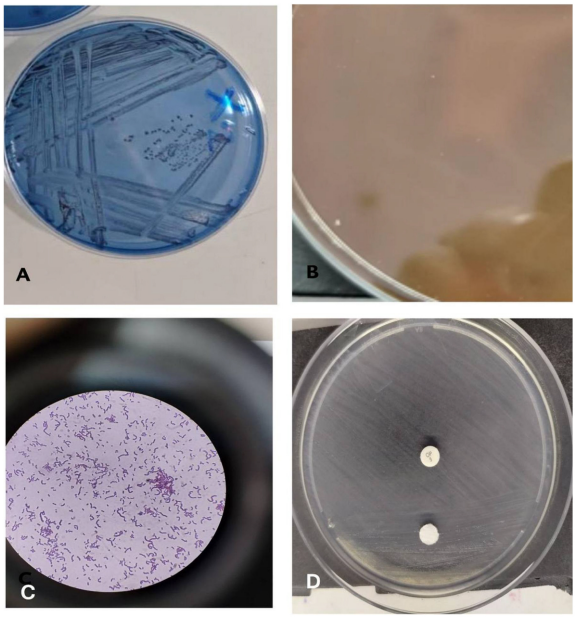

Fig. 1. A: Mitis Salivarius agar bluish, B: The colonies exhibited α-hemolysis (greenish) On blood agar. C: Gram-positive cocci arranged in chains, D: Optochin resistance.

However, molecular confirmation using PCR targeting the gtfR gene as evidenced by the presence of the specific 374 bp amplicon The statistical analysis revealed a highly significant difference between positive and negative findings, as shown by the P value (<0.0001), suggesting that the distribution of *S. oralis* among the tested samples is unlikely to have occurred by chance. as in Fig. 2 and Table 4.

3.2. HPLC

Based on the chromatographic analysis results of the sample titled "Flavonoids in Triphala," ten principal phenolic and flavonoid compounds were identified and their absolute quantities in milligrams were estimated. Leading the list in terms of quantity is Gallic Acid at 2058.837 mg, highlighting its prominent role as one of the core phenolic components in the

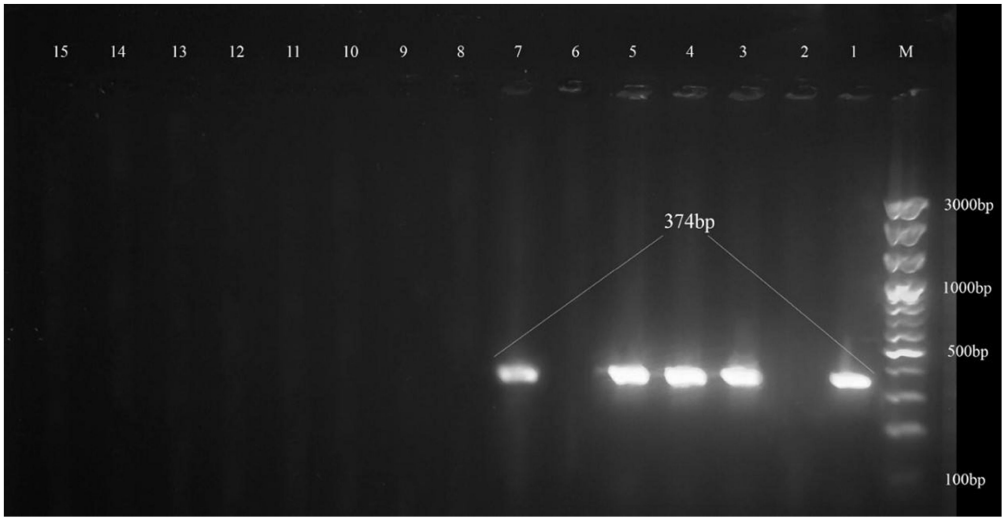

Fig. 2. PCR identification products for specific- gtfR region of Streptococcus oralis. Lanes: 1, 3-5, 7 are positive strains showed 374bp amplicon. Lane M: 100bp DNA ladder.

Table 4. PCR Identification of Streptococcus oralis isolates.

Results	Positive	Negative	Total
N	5	25	30
%	16.6	83.4	100%
P value	<0.0001*		

* Represent a significant difference at $p \leq 0.05$.

Triphala mixture. Following in order are Chebulagic Acid at 1548.336 mg, then Ellagic Acid at 1055.270 mg The analysis also revealed notable amounts of Chebulic Acid (811.606 mg), Rutin (219.140 mg), and Chlorogenic Acid (198.375 mg). The individual flavonoids were present in relatively lower quantities, with Catechin recorded at 108.708 mg, Quercetin at 112.701 mg, and Apigenin at 57.797 mg, while Kaempferol was the least among the named compounds at 55.775 mg.

The phytochemical composition of a commercial Aloe vera extract the results confirm the presence of characteristic anthraquinone derivatives, which are the primary bioactive markers associated with Aloe vera's pharmacological properties. The predominant compound identified was Aloin A/B (also known as barbaloin), eluting at a retention time of 5.03 minutes. Its estimated absolute quantity was determined to be 206.99 mg, constituting approximately 69.5% of the total quantified analyte mass in the injected sample. This finding aligns with the established literature, where aloin is reported as the principal anthrone C-glycoside in Aloe vera latex, responsible for its well-documented laxative effects. Two additional anthraquinone-related compounds were identified and quantified: Aloe-emodin (retention time 6.27 min, 21.58 mg, ~7.2%) and its aglycone, Emodin (retention time 10.14 min, 13.80 mg, ~4.6%). The presence of these compounds further authenticates the sample as a genuine Aloe vera extract and indicates a degree of processing, as free emodin is often a product of hydrolysis or degradation. An early-eluting, unidentified peak at 3.92 minutes accounted for 55.49 mg (~18.6%) of the total mass, which may correspond to phenolic acids, carbohydrates, or other polar constituents typical of the Aloe vera matrix. The combined mass of the quantified compounds totaled 297.86 mg, strongly suggesting that the analyzed material is a concentrated extract rather than a crude preparation. Compound identification was rigorously supported by comparing sample retention times with those of pure reference standards analyzed under identical chromatographic conditions (Aloin A/B: 5.08 min, Aloe-emodin: 6.09 min, Emodin: 10.18 min).

3.3. Antibacterial activity

In the present study, the antibacterial activity of *Triphala*, *Aloe vera* extract, and their combination was evaluated against S. oralis using MIC and MBC assays. The results demonstrated that *Triphala* exhibited an MIC at concentrations ranging between 5–10%, while its bactericidal activity (MBC) was observed at 20%. Similarly, the combination extract showed an identical pattern, with an MIC value of 5–10% and an MBC of 20%. In contrast, *Aloe vera* extract required notably higher concentrations to achieve antimicrobial efficacy, with an MIC of 60% and an MBC of 80% as in Fig. 5 and Table 5.

Table 5. MIC and MBC of Triphala, Aloe vera and combination extracts against S. oralis.

Extracts	MIC	MBC
Triphala	5–10 %	20%
Aloe vera,	60%	80%
combination	5–10%	20%

Table 6. The statistical analysis of S. oralis inhibition zone by different con. of aqueous Triphala, Aloe vera and combination extracts, CHX and D.W.

Agents	Con	N	Mean	±S.D.	ANOVA test
CHX	0.12%	4	13.75	0.50	
D.W		4	0.00	0.00	
Triphala Extract	20%	4	16.50	0.57	F = 764.667
	40%	4	18.50	0.57	Df = 20
	60%	4	20.25	0.50	P = 0.00, H. S
	80%	4	20.75	0.50	
	100%	4	22.75	0.50	
CHX	0.12%	4	13.75	0.50	
D.W		4	0.00	0.00	F = 374.211
Aloe vera Extract	20%	4	0.00	0.00	Df = 20
	40%	4	0.00	0.00	P = 0.00, H. S
	60%	4	12.00	1.4421	
	80%	4	16.75	1.25	
	100%	4	18.25	0.95	
CHX	0.12%	4	13.75	0.50	
D.W		4	0.00	0.00	
Combination Extract	20%	4	18.75	0.50	F = 329.704
	40%	4	20.75	1.50	Df = 20
	60%	4	22.50	1.00	P = 0.00, H. S
	80%	4	23.50	1.00	
	100%	4	25.25	0.50	

However, Table 6 presents the statistical analysis of the inhibition zones of Streptococcus oralis treated with different concentrations of aqueous Triphala, Aloe vera, and their combination extracts, in comparison with 0.12% CHX and distilled water (D.W.) as controls. For Triphala extract, the inhibition zone increased progressively with increasing concentration, starting from 16.50 mm at 20% and reaching 22.75 mm at 100%. The ANOVA test revealed a highly significant difference among the concentrations ($F = 764.667$, $df = 20$, $P = 0.00$, H.S), indicating that concentration has a strong effect on antibacterial activity. Triphala at higher concentrations (80–100%) showed inhibition zones greater than CHX (13.75 mm). For Aloe vera extract, lower concentrations (20% and 40%) showed no inhibitory effect (0.00 mm). Activity started at 60% (12.00 mm) and increased to 18.25 mm at 100%. The ANOVA test for Aloe vera also showed highly significant differences among concentrations ($F = 374.211$, $df = 20$, $P = 0.00$, H.S). Higher concentrations (≥80%) demonstrated superior activity compared with CHX. Regarding the combination extract, all concentrations displayed strong antibacterial activity, starting from 18.75 mm at 20% and reaching 25.25 mm at 100%. The inhibition zones were higher than those of Triphala and Aloe vera alone at corresponding concentrations. ANOVA again indicated a highly significant difference among groups ($F = 329.704$, $df = 20$, $P = 0.00$, H.S)

Table 7 illustrates the LSD post-hoc comparison between the mean inhibition zones of *Streptococcus oralis* produced by different concentrations of aqueous Triphala, Aloe vera, and their combination extracts in relation to 0.12% CHX and distilled water. The results show that all concentrations of Triphala extract exhibited highly significant differences compared with CHX ($P = 0.00$), with negative mean differences indicating that Triphala produced larger inhibition zones than CHX across all tested concentrations. Similarly, Triphala demonstrated highly significant superiority over distilled water, reflecting its strong and concentration-dependent antibacterial activity, which reached its maximum effect at 100%. In contrast, Aloe vera extract showed minimal or no antibacterial effect at low concentrations (20% and 40%), where the inhibition zones were significantly smaller than those of CHX. The activity began to appear at 60%, showing highly significant differences from both CHX and D.W., while the 80% concentration showed no significant difference from CHX, indicating comparable activity. At 100%, Aloe vera demonstrated significantly greater antibacterial action than CHX ($P = 0.00$). The combination extract displayed the highest antibacterial effectiveness among all treatments, with all its concentrations showing highly significant differences from CHX and D.W. and consistently producing larger inhibition zones. Its effect increased progressively with concentration, reaching the maximum at 100%, which recorded the largest mean difference relative to CHX and D.W. Overall, the LSD analysis confirms that Triphala and the combination extracts possess strong antibacterial activity that surpasses CHX, while Aloe vera shows marked activity only at higher concentrations, with the combination extract being the most potent treatment across all tested concentrations.

Table 8 presents the LSD post-hoc comparison among different concentrations of aqueous Triphala, Aloe vera, and the combination extracts to evaluate the concentration-dependent variations in their inhibitory effects against *Streptococcus oralis*. For the Triphala extract, the results show a consistent and highly significant increase in antibacterial activity with increasing concentration, as all comparisons between 20%, 40%, 60%, 80%, and 100% yielded highly significant differences ($P = 0.00$), except for the comparison between 60% and 80%, which was not significant, indicating that these two concentrations produce nearly similar effects. Overall, Triphala

Table 7. Comparison of mean value of S. oralis inhibition zone by between each aqueous Triphala, Aloe vera and combination extracts with CHX and D.W by LSD.

	CHX			D.W			
Extract	Conc	Mean differences	Sign	Desc	Mean	P-value	Desc
Triphala Extract	20%	–2.75	0.00	H. S	–16.50	0.00	H.S
	40%	–4.75	0.00	H. S	–18.50*	0.00	H.S
	60%	–6.50	0.00	H. S	–20.25-*	0.00	H.S
	80%	–7.00	0.00	H.S	–20.75	0.00	H.S
	100%	–9.00	0.00	H.S	–22.75-*	0.00	H.S
Aloe vera Extract	20%	13.75*	0.00	H.S	0.00	1.00	N.S
	40%	13.75*	0.00	H.S	0.00	1.00	H.S
	60%	1.75	0.00	H.S	–12.00	0.00	H.S
	80%	–3.00	0.117	N.S	–16.75	0.00	H.S
	100%	–4.50	0.00	H.S	–18.25	0.00	H.S
Combination Extract	20%	–5.00	0.00	H.S	–18.75-*	0.00	H.S
	40%	–7.00	0.00	H.S	–20.75	0.00	H.S
	60%	–8.75	0.00	H.S	–22.50	0.00	H.S
	80%	–9.700	0.00	H.S	–23.50	0.00	H.S
	100%	–11.50	0.00	H.S	–25.25	0.00	H.S

Table 8. Comparison of mean value of S.oralis inhibition zone by between each aqueous Triphala, Aloe vera and combination extracts by LSD.

	Triphala Extract			Aloe vera Extract			Combination Extract		
CONC.	Mean differences	P value	Desc	Mean differences	P value	Desc	Mean differences	P value	Desc
20%									
40%	–2.00	0.00	H.S	0.00	1.00	N.S	–2.00	0.00	H.S
60%	–3.75	0.00	H.S	–12.00	0.00	H.S	–3.75	0.00	H.S
80%	–4.25	0.00	H.S	–16.75	0.00	H.S	–4.75	0.00	H.S
100%	–6.250	0.00	H.S	–18.25	0.00	H.S	–6.50	0.00	H.S
40%									
60%	–1.75	0.00	H.S	–12.00	0.00	H.S	–1.75	0.01	H.S
80%	–2.25	0.00	H.S	–16.75	0.00	H.S	–2.75	0.00	H.S
100%	–4.25	0.00	H.S	–18.25	0.00	H.S	–4.50	0.00	H.S
60%									
80	–0.50	0.181	N.S	–4.75	0.00	H.S	–1.00	0.12	N.S
100%	–2.50	0.00	H.S	–6.25	0.00	H.S	–2.75	0.00	H.S
80%									
100%	2.00	0.00	H.S	–1.50	0.02	H.S	–1.75	0.01	H.S

demonstrates a clear dose–response pattern, with higher concentrations producing larger inhibition zones. In contrast, Aloe vera extract exhibited a distinctly different behavior. The comparisons showed no significant difference between 20% and 40% (P = 1.00), confirming the absence of antibacterial activity at these low concentrations. However, as the concentration increased, Aloe vera revealed highly significant differences in all subsequent comparisons, demonstrating a strong and steep increase in activity between 60% and 100%, with the largest differences occurring between lower and higher concentrations (e.g., 20% vs. 100% and 40% vs. 100%). This reflects the concentration-dependent nature of Aloe vera's antibacterial effect, which becomes evident only at higher concentrations. Regarding the combination extract, most concentration comparisons showed highly significant differences (P = 0.00), indicating a strong and progressive increase in antibacterial activity as the concentration increased. Similar to Triphala, the comparison between 60% and 80% was not significant, suggesting comparable inhibitory effects at these two levels. However, the differences between low and high concentrations, especially between 20% and 100%, were highly significant, confirming that the combination extract exhibits a potent and concentration-dependent antibacterial activity.

Table 9 shows the descriptive statistics and T-test comparison between Triphala and Aloe vera extracts at the same concentrations to evaluate differences in their antibacterial activity against *Streptococcus oralis*. The results demonstrate that Triphala extract produced significantly larger inhibition zones than Aloe vera at all tested concentrations. At low concentrations (20% and 40%), Triphala exhibited substantial antibacterial activity with mean inhibition zones of 16.50 mm and 18.50 mm, respectively, while Aloe vera showed no inhibition (0.00 mm), resulting in

Table 9. Descriptive statistics and comparisons between mean value of S. oralis inhibition zone for same conc of Triphala Extract and Aloe vera Extract by T test.

Descriptive Statistics			Descriptive Statistics				
Triphala Extract			Aloe vera Extract		Descriptive Statistics		
CONC	Mean	SD	Mean	SD	T test	P.value	Desc
20%	16.50	0.57	0.00	0.00	41.64	0.00	H.S
40%	18.50	0.57	0.00	0.00	64.08	0.00	H.S
60%	20.25	0.50	12.00	1.44	13.11	0.00	H.S
80%	20.75	0.50	16.75	1.25	4.89	0.016	H.S
100%	22.75	0.50	18.25	0.95	9.00	0.003	H.S

Table 10. Descriptive statistics and comparisons between mean value of S. oralis inhibition zone for same conc of Triphala Extract and Combination Extract by T test.

Descriptive Statistics			Descriptive Statistics				
Triphala Extract			Combination Extract		Descriptive Statistics		
CONC	Mean	SD	Mean	SD	T test	P.value	Desc
20%	16.50	0.57	18.75	0.50	–9.00	0.003	H.S
40%	18.50	0.57	20.75	1.50	-4.70-	0.00	H.S
60%	20.25	0.50	22.50	1.00	0.72	0.018	H.S
80%	20.75	0.50	23.50	1.00	-5.74-	0.10	N.S
100%	22.75	0.50	25.25	0.50	-8.66-	0.003	H.S

extremely high T-values (41.64 and 64.08) and highly significant differences (P = 0.00). At 60%, both extracts exhibited activity; however, Triphala remained markedly superior (20.25 mm vs. 12.00 mm), producing a highly significant difference (T = 13.11, P = 0.00). At 80%, the gap between the two extracts narrowed, with Triphala showing 20.75 mm compared to 16.75 mm for Aloe vera, yet the difference remained statistically significant (P = 0.016). Even at 100%, where both extracts reached their maximum activity, Triphala continued to demonstrate significantly greater inhibition (22.75 mm vs. 18.25 mm; T = 9.00, P = 0.003).

Table 10 presents the descriptive statistics and T-test comparisons between Triphala and the combination extract at identical concentrations to determine which treatment exhibits greater antibacterial activity against *Streptococcus oralis*. The results demonstrate that the combination extract consistently produced larger inhibition zones than Triphala across almost all concentrations. At 20%, the combination extract showed significantly greater activity (18.75 mm) compared with Triphala (16.50 mm), resulting in a highly significant difference (P = 0.003). A similar pattern was observed at 40%, where the combination extract achieved 20.75 mm versus 18.50 mm for Triphala, again with a highly significant difference (P = 0.00). At 60%, both extracts exhibited strong activity, yet the combination extract remained superior (22.50 mm vs. 20.25 mm), and the difference was statistically significant (P = 0.018). At 80%, although the combination extract continued to show a larger inhibition zone (23.50 mm compared with 20.75 mm), the difference was not statistically significant (P = 0.10), suggesting that at this concentration, both extracts exert comparable antibacterial effects. At 100%, the combination extract reached its highest activity (25.25 mm), significantly exceeding the inhibition produced by Triphala (22.75 mm), with a highly significant difference (P = 0.003).

Table 11 presents the descriptive statistics and T-test comparison between Aloe vera and the combination extract at identical concentrations to assess the differences in their antibacterial activity against *Streptococcus oralis*. The results clearly demonstrate that the combination extract exhibits significantly greater inhibitory effects compared with Aloe vera across all tested concentrations. At 20% and 40%, Aloe vera showed no antibacterial activity (0.00 mm), while the combination extract produced inhibition zones of 18.75 mm and 20.75 mm, respectively, yielding extremely high T-values and highly significant differences (P = 0.00). Even at 60%, where Aloe vera first demonstrated measurable activity (12.00 mm), the combination extract remained markedly superior (22.50 mm), with the difference being highly significant (P = 0.00). This pattern continued at higher concentrations, as Aloe vera at 80% and 100% produced inhibition zones of 16.75 mm and 18.25 mm, respectively, whereas the combination extract achieved substantially larger zones of 23.50 mm and 25.25 mm, with all comparisons showing highly significant differences.

Table 11. Descriptive statistics and comparisons between mean value of S. oralis inhibition zone for same conc of Aloe vera and Combination Extract by T test.

Descriptive Statistics			Descriptive Statistics				
Aloe vera			Combination Extract		Descriptive Statistics		
CONC	Mean	SD	Mean	SD	T test	P.value	Desc
20%	0.00	0.00	18.75	0.50	–77.00	0.00	H.S
40%	0.00	0.00	20.75	1.50	–27.66	0.00	H.S
60%	12.00	1.44	22.50	1.00	–36.37	0.00	H.S
80%	16.75	1.25	23.50	1.00	–9.00	0.003	H.S
100%	18.25	0.95	25.25	0.50	–17.14–	0.000	H.S

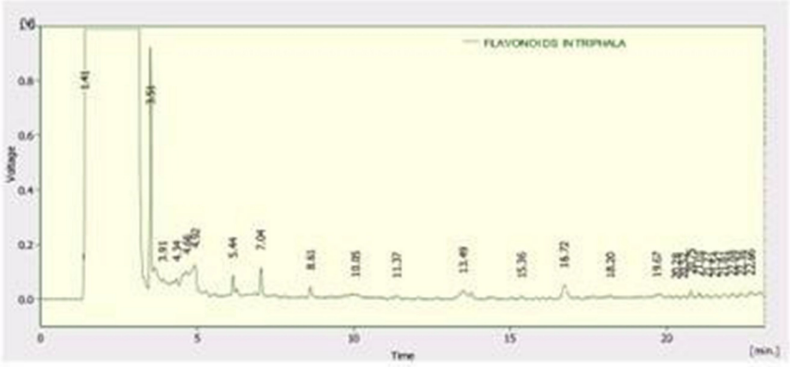

Fig. 3. The phytochemical composition of Triphala aqueous extract.

4. Discussion

The present study evaluated the antibacterial activity of aqueous extracts of Triphala, Aloe vera, and their combination against Streptococcus oralis, a clinically important early colonizer of dental plaque. The results demonstrated that 16.6% of the samples were classified as positive for S. oralis, indicating confirmed bacterial presence, whereas 83.4% of the samples were negative. These findings are in agreement with previous studies reporting a high prevalence of S. oralis in dental caries patients. Al-Qazzaz et al. identified *S. oralis* isolates from both diabetic and non-diabetic dental caries patients using phenotypic and molecular methods, including Gram staining, API 20 Strep, hemolysis patterns on blood agar, and PCR analysis targeting the gtfR gene, which yielded a specific 374-bp product confirming bacterial identity in saliva, buccal swabs, and cultured isolates.[36] Similarly, Banas et al. reported the isolation of S. oralis strains from the majority of dental caries patients and demonstrated extensive genetic diversity using arbitrary primed PCR analysis.[37] Furthermore, Morales-Dorantes et al. highlighted the frequent occurrence of S. oralis among mitis-group streptococci isolated from dental plaque and emphasized its potential role as a reservoir for antibiotic resistance genes.[38] Since early bacterial adhesion is a prerequisite for colonization and biofilm formation, strategies targeting bacterial attachment mechanisms are considered effective approaches for controlling biofilm-associated infections.[39] In this context, surface appendages such as pili or fimbriae play a crucial role in bacterial adhesion. Sortase-dependent pili, which are exclusive to Gram-positive bacteria, have been identified in early dental plaque colonizers including S. oralis and S. sanguinis, contributing significantly to their pathogenic potential.[40,41]

Based on the chromatographic analysis presented in Fig. 3, ten major phenolic and flavonoid compounds were identified in the Triphala extract. Among these compounds, gallic acid, chebulagic acid, and ellagic acid were the most abundant constituents, while the remaining compounds were detected in moderate to low concentrations, with kaempferol showing the lowest level. These findings are consistent with previous reports by Charoenchai et al., who demonstrated that ascorbic acid, gallic acid, corilagin, chebulagic acid, and chebulinic acid were sequentially eluted in Triphala extracts using HPLC analysis.[42] The gallic acid content of Triphala and modified Triphala formulations ranged from 2.66–2.87% w/w, compared with 1.32–4.02% w/w in the individual herbal components, while rutin content ranged from 0.65–2.94% w/w compared with 0.61–2.61% w/w in the individual herbs.[42] Furthermore, electrospray mass spectrometry analysis confirmed the presence of ascorbic acid, gallic acid,

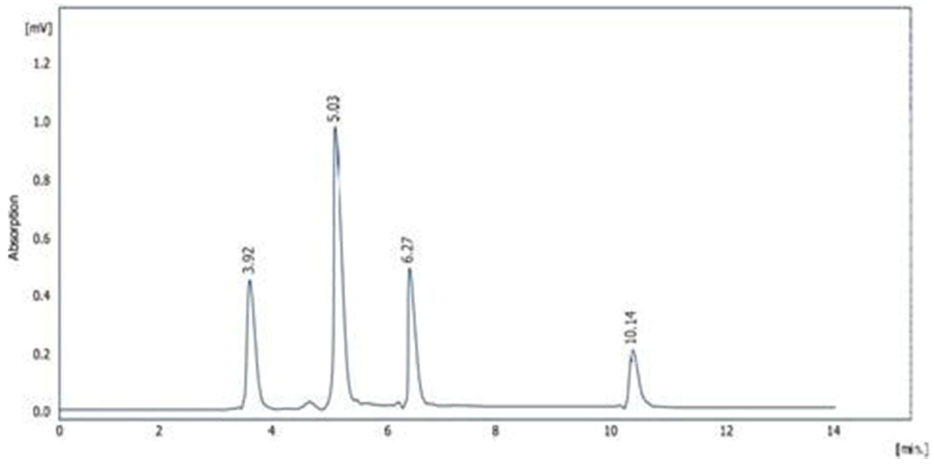

Fig. 4. The phytochemical composition of Aloe vera aqueous extract.

corilagin, chebulagic acid, rutin, chebulinic acid, and trace amounts of quercetin in Triphala preparations.[42] Five phenolic compounds—gallic acid, corilagin, chebulagic acid, rutin, and chebulinic acid—were therefore selected as standard chemical markers for Triphala characterization. Previous studies have demonstrated that HPLC is a reliable and essential analytical tool for the qualitative and quantitative evaluation of chemical constituents in Triphala formulations, with gallic acid consistently identified as the major compound.[43] In addition, it was reported that gallic acid was the predominant constituent in Terminalia chebula and Phyllanthus emblica, while chebulagic acid was the major compound in Terminalia bellirica. However, rutin was not detected in all tested formulations, leading to the quantification of only gallic acid, corilagin, chebulagic acid, and chebulinic acid in subsequent analyses.[43]

In addition, the chromatographic profile of Aloe vera presented in Fig. 4 confirmed the presence of characteristic anthraquinone derivatives in the aqueous Aloe vera extract. Aloin A/B was identified as the predominant compound, accounting for approximately 69.5% of the total detected constituents, followed by aloe-emodin and emodin. These findings are consistent with those reported by Numan, who demonstrated through HPLC analysis that Aloe vera gel contains a wide range of phenolic compounds, including cinnamic acid, aloin, aloe-emodin, aloetic acid, anthranol, and sinapic acid, detected at various retention times with considerable peak areas.[44] In the same study, several flavonoids such as quercetin, kaempferol, and rutin were also identified, confirming the complex phytochemical profile of Aloe vera.[44]

Furthermore, Wariyah et al. reported detectable levels of myricetin, quercetin, and kaempferol in Aloe vera extracts, with kaempferol being the most abundant flavonoid among those identified.[45] López et al. demonstrated that catechin, sinapic acid, gentisic acid, epicatechin, kaempferol, apigenin, and gallic acid were the predominant compounds in Aloe vera leaf extracts, whereas gentisic acid, epicatechin, and quercetin were more abundant in the gel extract.[46] Similarly, Laib et al. confirmed that phenolic acids and flavonoids, particularly epicatechin and quercetin, constituted the major bioactive components of Aloe vera gel.[47] Collectively, these findings support the current chromatographic results and highlight the richness of Aloe vera in biologically active phenolic and flavonoid compounds.

Regarding the antibacterial activity of the aqueous extracts, no previous studies have specifically investigated the antimicrobial effects of Aloe vera or Triphala against Streptococcus oralis. Therefore, the present findings fill an important gap in the existing literature. In interpreting these results, it is appropriate to compare them with previous studies that evaluated the antibacterial activity of these extracts against other oral microorganisms, particularly Streptococcus mutans and related oral pathogens.

The present findings are in agreement with those reported by Laib et al., who investigated the antibacterial effect of Aloe vera gel against several oral pathogens, including Actinobacillus actinomycetemcomitans, Clostridium bacilli, Streptococcus mutans, and Staphylococcus aureus.[47] In that study, Aloe vera gel exhibited significant antibacterial activity at 100% and 50% concentrations ($t = 7.504$, $p < 0.001$), whereas no inhibitory effect was observed at lower concentrations. At 100% concentration, the inhibition zones measured were 6.9 mm for A. actinomycetemcomitans, 6.3 mm for C. bacilli, 6.8 mm for S. mutans, and 6.6 mm for S. aureus, supporting the concentration-dependent antibacterial activity of Aloe vera.

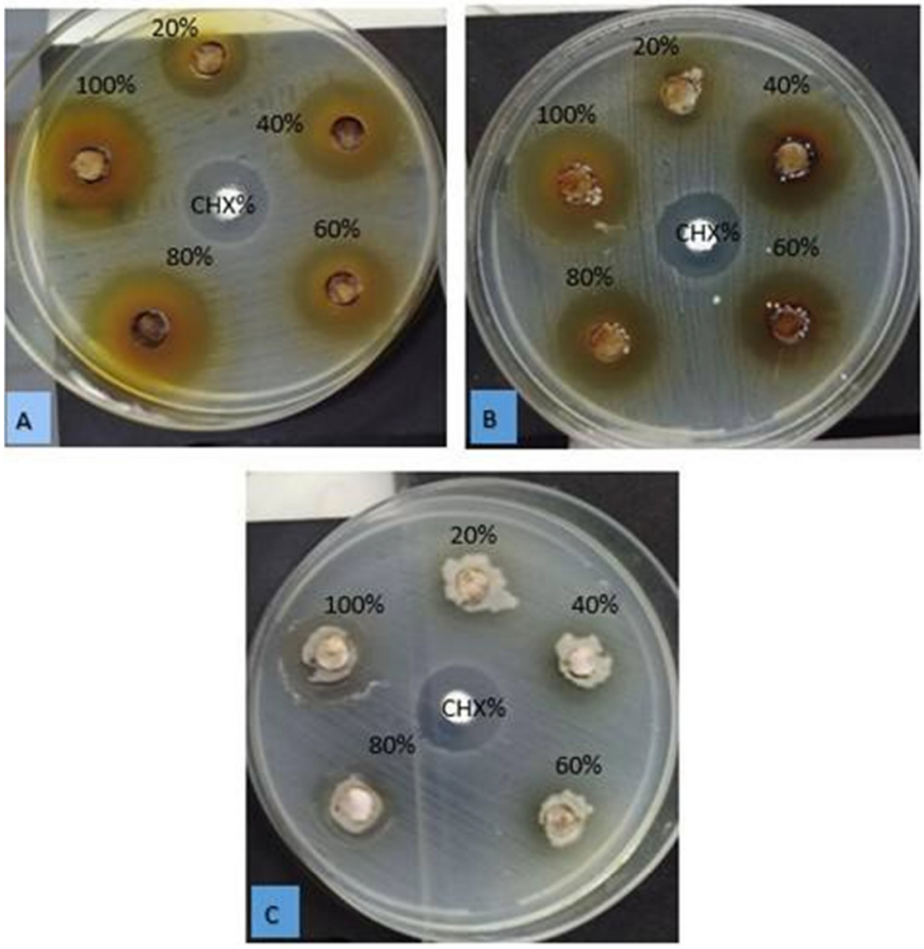

Fig. 5. The antibacterial activity of Aqueous extract against S. oralis. A: Combination extract. B: Triphala extract: Aleo vera extract.

Similarly, Naghsh et al. evaluated the antimicrobial efficacy of three mouthwashes—chamomile (Matrika), Aloe vera–green tea, and chlorhexidine (CHX)—with distilled water serving as a control, against five oral bacterial species, including *Streptococcus oralis*, *Streptococcus sanguinis*, and *Streptococcus mutans* as primary colonizers, as well as *Porphyromonas gingivalis* and *Eikenella corrodens* as secondary colonizers.[48] Colony-forming unit counts, disc diffusion, and well diffusion methods were employed to assess antibacterial activity. The results demonstrated that CHX exhibited a significantly higher antibacterial effect than the herbal mouthwashes across all tested methods ($p < 0.001$). Nevertheless, both herbal mouthwashes showed statistically significant antibacterial activity against all tested bacterial species ($p < 0.001$). Moreover, the Aloe vera–green tea mouthwash displayed significantly greater antibacterial activity than chamomile mouthwash against all bacterial species, except *S. sanguinis* ($p < 0.05$). These findings further support the antibacterial potential of Aloe vera-based formulations against oral *streptococci*, including *S. oralis*.

In vitro experiments reported by Abbas demonstrated a statistically significant reduction in the viable count of Streptococcus mutans at all tested concentrations of alcoholic Aloe vera extract, with increasing extract concentration leading to greater reductions in bacterial viability.[49] In contrast, the present study demonstrated a significant reduction in the viable count of S. mutans only at higher concentrations (38% and 48%) of the aqueous Aloe vera extract. Moreover, the diameter of inhibition zones against S. mutans was highly significant at all concentrations of the alcoholic extract when compared with the aqueous extract, indicating that alcoholic extraction may enhance the antibacterial efficacy of Aloe vera. These findings suggest that the solvent used for extraction plays a critical role in determining the antimicrobial potency of Aloe vera preparations.

In contrast to in vitro findings, in vivo studies have reported variable antimicrobial effects of Aloe vera.

Komchornrit et al. evaluated the antimicrobial efficacy of an Aloe vera–based mouthwash against key cariogenic pathogens in adults with dental caries and compared its effects with a standard chlorhexidine (CHX) mouthwash.[50] The results demonstrated that the Aloe vera mouthwash group showed a statistically significant reduction in salivary Candida levels ($P = 0.008$). Although a reduction in Streptococcus mutans counts was observed, this decrease did not reach statistical significance ($P = 0.096$). In contrast, the CHX group exhibited significant reductions in both S. mutans ($P = 0.046$) and Candida species ($P = 0.002$). Neither group showed a significant change in Lactobacilli levels, and no significant differences in microbial counts were detected between the two groups after the 7-day intervention period.

The antimicrobial activity of Aloe vera is believed to be mediated through multiple bioactive compounds that exert synergistic effects on microbial cells. Anthraquinones, such as aloin and emodin, can penetrate bacterial cells and inhibit protein synthesis by binding to ribosomal subunits, leading to disruption of essential cellular functions.[51] In addition, saponins possess direct antibacterial properties by damaging bacterial cell membranes, while polysaccharides are thought to enhance host immune responses, facilitating microbial clearance. These combined mechanisms may explain the antimicrobial potential of Aloe vera observed in both in vitro and in vivo studies.

On the other hand, the antibacterial activity of Triphala observed in the present study is consistent with findings reported by Gupta et al., who evaluated the antimicrobial efficacy of both ethanolic and aqueous extracts of Triphala against oral streptococci.[52] In that study, the ethanolic extract inhibited Streptococcus mutans, Streptococcus sanguinis, and Streptococcus salivarius at minimum inhibitory concentrations (MICs) of 1 mg/ml, 0.5 mg/ml, and 0.5 mg/ml, respectively. Similarly, the aqueous extract exhibited MIC values of 1 mg/ml for S. mutans, 0.5 mg/ml for S. sanguinis, and 2 mg/ml for S. salivarius.[52] Antimicrobial activity was assessed using the agar well diffusion method at concentrations of 6.25%, 12.5%, 25%, and 50%, with 0.2% chlorhexidine used as a positive control and dimethyl sulfoxide (DMSO) as a negative control. Both ethanolic and aqueous extracts of Triphala demonstrated concentration-dependent inhibition of the tested streptococcal species, with maximum antibacterial activity observed at the 50% concentration. Importantly, no statistically significant difference was reported between the ethanolic and aqueous extracts in terms of the mean diameter of inhibition zones against S. mutans, indicating comparable antimicrobial efficacy of the two extraction methods.[52]

Paulraj and Nagar investigated the antimicrobial activity of aqueous Triphala extract and ethanolic propolis extract and reported inhibition of Streptococcus mutans and Lactobacillus species at concentrations of 0.15 mg/mL and 0.10 mg/mL for Triphala, and 0.025 mg/mL and 0.022 mg/mL for propolis, respectively.[53] Their study demonstrated superior antimicrobial efficacy of Triphala- and propolis-modified glass ionomer cement against both bacterial strains. Similarly, Biradar et al. reported that Triphala effectively retards bacterial growth, supporting its antimicrobial potential.[53]

In an in vivo study, Srinagesh et al. evaluated the effect of a 6% Triphala mouthwash on salivary streptococcal levels following twice-daily use for 48 hours and 7 days, and compared its efficacy with 0.2% chlorhexidine.[54] The results showed that the Triphala group exhibited a 17% and 44% reduction in salivary streptococci at 48 hours and 7 days, respectively, while the chlorhexidine group demonstrated a 16% and 45% reduction over the same period ($P < 0.001$). Notably, the reduction in CFU/mL observed in the Triphala group closely paralleled that of the chlorhexidine group, indicating comparable antimicrobial efficacy.

The antimicrobial properties of Triphala are attributed to its rich phytochemical composition, including phenolic acids, flavonoids, and tannins. Phytochemical analyses using HPLC, Folin–Ciocalteu, and Folin–Denis methods have revealed that Triphala contains approximately 35% tannins and 40% polyphenols, with a particularly high gallic acid content, making it an excellent candidate for in vivo antimicrobial applications.[55] These bioactive compounds are known to inhibit both Gram-positive and Gram-negative bacteria by disrupting bacterial cell walls or interfering with cell division.[56] In particular, tannic acid has been reported to exert bacteriostatic or bactericidal effects against several pathogenic microorganisms.[57] These mechanisms may explain the enhanced antimicrobial activity observed in the present study. Furthermore, Srinagesh et al. concluded that the anti-oral streptococcal efficacy of Triphala is comparable to that of chlorhexidine,[54] a finding further supported by Prabhakar et al., who demonstrated that Triphala exhibited significantly higher antibacterial activity than chlorhexidine in vitro.[58]

One of the major limitations of the present study is that the individual bioactive constituents within the tested plant extracts were neither isolated nor purified, and therefore were not evaluated independently. Consequently, it was not possible to determine which specific compound, or combination of compounds, was primarily responsible for the observed

antibacterial activity against *Streptococcus oralis*. The results thus represent the cumulative effect of the crude extracts rather than the distinct contribution of individual phytochemical components. Future investigations employing comprehensive phytochemical fractionation, isolation, and independent evaluation of purified compounds are necessary to elucidate their specific antibacterial roles and underlying mechanisms of action.

Those studies generally reported inhibitory effects, supporting the antimicrobial potential observed in the present work; however, species-specific differences and methodological variations should be taken into account when interpreting such comparisons. The findings of the current study demonstrated that the combination extract exhibited the highest inhibitory activity against Streptococcus oralis, followed by Triphala alone, whereas Aloe vera required relatively high concentrations to produce measurable antibacterial effects. These results indicate a clear synergistic interaction between Triphala polyphenols and Aloe vera anthraquinones, which is consistent with the phytochemical richness revealed by HPLC profiling. Moreover, the strong antibacterial activity of Triphala observed in this study—even at low concentrations (MIC 5–10%, MBC 20%), as shown in Table 5—further supports its potential as an effective natural antimicrobial agent.

5. Conclusion

The results of the present study demonstrate that Triphala and Aloe vera exhibit significant antibacterial activity against *S. oralis*, with the combination extract showing the strongest effect. These findings are fully supported by previous studies and by the phytochemical composition identified through HPLC. Together, they suggest that the Triphala–Aloe vera combination is a promising natural antimicrobial agent for early plaque control and could serve as an effective alternative to conventional chemical mouthrinses.

Highlights

- Aqueous Triphala and Aloe vera extracts showed antibacterial activity against S. oralis.
- The combination extract produced the strongest inhibition and lowest MIC/MBC values.
- Synergistic effects were linked to complementary polyphenolic and anthraquinone actions.
- HPLC confirmed high levels of gallic, chebulagic, chebulinic acids and Aloe anthraquinones.
- The herbal combination may serve as a natural alternative to chlorhexidine for plaque control.

Conflicts of interest

The author declares no conflict of interests.

Disclaimer

None.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author used ChatGPT Open AI in order to improve readability and language of the work. After using this tool/service, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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Ethical apporoval

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Author contributions

A.H. conceived the study, performed laboratory work, analyzed data, and drafted the manuscript. M.A.A.A. supervised the research, critically revised the manuscript, and approved the final version.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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