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Comparative evaluation of antibacterial activity of cranberry (vaccinium macrocarpon) extract, cinnamon (cinnamomum zeylanicum) extract and xylitol against Streptococcus Mutans - An in-vitro study.

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ABSTRACT

Background:
Early Childhood Caries (ECC) is a prevalent and aggressive form of dental caries affecting children under six years. *Streptococcus mutans* is a key pathogen in ECC, largely due to its role in biofilm formation and acid production. Natural antimicrobials such as cranberry, cinnamon, and xylitol have shown individual potential against *S. mutans*, but comparative data is lacking.
Aim:
To evaluate and compare the antibacterial activity of cranberry extract, cinnamon extract, and xylitol against *Streptococcus mutans* using an in-vitro biofilm model.
Materials and methods
An in-vitro study was conducted using a standard strain of *Streptococcus mutans*. Three test groups were prepared using cranberry extract, cinnamon extract, and xylitol, respectively. The antibacterial activity was assessed by measuring the zone of inhibition, colony-forming unit (CFU) counts, and biofilm inhibition percentage. Data were analyzed using one-way ANOVA followed by post hoc tests.
Results
Cranberry extract showed the highest antibacterial activity with the largest zone of inhibition and greatest reduction in CFU counts and biofilm formation. Cinnamon extract demonstrated moderate activity, while xylitol showed minimal antibacterial effects in vitro. Statistically significant differences were observed among the three groups ($p < 0.05$).
Conclusion:
Cranberry and cinnamon extracts demonstrated superior antibacterial efficacy against *Streptococcus mutans* compared to xylitol in an in-vitro model. These natural agents may offer potential as adjuncts in caries prevention strategies, warranting further clinical evaluation.

INTRODUCTION

Early Childhood Caries (ECC) is the presence of one or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger. Severe ECC is any sign of smooth surface caries in a child younger than 3 years of age, one or more cavitated, missing(due to caries), or filled smooth surfaces in primary maxillary anterior teeth in ages 3 to 5yrs, or a decayed, missing or filled score of > 4(age 3), > 5(age 4), or > 6(age 5).[1] The prevelance of ECC is very high in India, Andhra Pradesh was found to have the highest prevalence of ECC at 63%, and the lowest prevalence was reported in Sikkim (41.92%). [2] ECC is a result of complex, dynamic interactions between microorganisms, host and diet, leading to the establishment of highly pathogenic biofilms. Biofilm matrix plays a key role in the pathogenesis of dental caries, particularly when conditions (e.g., sugar-laded dietary behavior) are conducive to the development of ECC. The matrix provides an essential physical-scaffold that facilitate microbial accumulation and adherence onto teeth while providing a diffusion-limiting milieu that help to create low pH microenvironments at the biofilm/tooth surface interface. Mutans streptococci, particularly S. mutans, are major organisms involved in the etiology and pathogenesis of ECC.[3]

Streptococcus mutans is a facultative anaerobic, gram-positive coccus which is considered a key pathogen in dental caries. S. mutans- derived glucosyltransferase (GTF) exoenzymes assemble the exopolysaccharide (EPS) glucan-rich matrix scaffold, which confers structural integrity and bulk to plaque biofilms, enhances microbial adhesion and co-aggregation, and provides a readily metabolizable carbohydrate source for sustained acid production.[4]Cranberry (Vaccinium macrocarpon), a native plant of North America, has been recognized to have several properties which may confer health benefits on humans. They are grown in American continent, European continent and parts of India. They are rich sources of bioactive flavinoids including flavinols, anthocyanins and proanthocyanidins offering a significant therapeutic potential for biofilm-related diseases like ECC.5 The bark of various cinnamon species is one of the most important and popular spices used worldwide not only for cooking but also in traditional and modern medicine .6The most important constituents of cinnamon are cinnamaldehyde, trans-cinnamaldehyde, procyanidins and catechins which are responsible for its biological activities.[7]Xylitol, a five-carbon non-cariogenic natural sugar alcohol, has shown potential in caries prevention in children by interfering with MS colonization. Xylitol-wipe use has been shown to be safe and well-accepted by both parents and infants. [8] Objectives of the study was to evaluate the inhibitory effect of cinnamon extract, cranberry extract, and xylitol on *Streptococcus mutans* in an in vitro biofilm model, and to compare the percentage inhibition of *S. mutans* among the three agents in the same model.

Despite the widespread use of antimicrobial agents in oral care, the emergence of resistance and undesirable side effects associated with conventional therapies highlight the need for alternative, natural compounds. While cranberry (*Vaccinium macrocarpon*) and cinnamon (*Cinnamomum zeylanicum*) extracts, as well as xylitol, have individually shown promise in inhibiting *Streptococcus mutans*, limited comparative data exist evaluating their efficacy within an in vitro biofilm model. This study aims to address this gap by systematically comparing the antibacterial potential of these three agents against *S. mutans*, thereby contributing to evidence-based development of preventive strategies for dental caries.

Materials and Methods

Biofilm Formation

A standard *Streptococcus mutans* strain (MTCC 497) was used to develop the biofilm model. Luria-Bertani (LB) broth was prepared using tryptone (10 g), sodium chloride (10 g), and yeast extract (6 g) in 1000 ml of distilled water. The medium was autoclaved at 121°C for 15 minutes. The bacterial strain was inoculated into 30 ml of LB broth and incubated in sterile 96-well microtiter plates at 37°C for 48 hours to allow biofilm formation (**Figure 2**).

Sample Preparation

Three agents were evaluated:

- **Sample 1**: Cranberry (*Vaccinium macrocarpon*) extract
- **Sample 2**: Cinnamon (*Cinnamomum zeylanicum*) extract
- **Sample 3**: Xylitol

Each sample (10 mg) was dissolved in 1 ml of dimethyl sulfoxide (DMSO). Serial dilutions were prepared by pipetting 10 µl (100 µg), 20 µl (200 µg), 30 µl (300 µg), 40 µl (400 µg), 50 µl (500 µg), and 60 µl (600 µg), and the final volume was adjusted to 60 µl with DMSO (Figure 1).

Microtiter Plate Assay

To prevent edge effects, 300 µl of sterile deionized water was added to the peripheral wells of the 96-well plate. All remaining wells received 100 µl of sterile LB broth.

- Blank wells: without organism and test agent.
- Control wells: containing *S. mutans* only.
- Test wells: received 100 µl of *S. mutans* inoculum and the appropriate volume of the test agent to reach final concentrations of 100 µg to 600 µg, adjusted to 200 µl with normal saline.

After 48 hours of incubation at 37°C, contents were discarded, and wells were washed with saline. Biofilms were stained with 10 µl of 0.1% crystal violet and incubated for 30 minutes at 37°C. Excess stain was rinsed off, and absorbance was recorded at 620 nm using a spectrophotometer (Figures 3 and 4).

Results

Statistical Analysis

All data were compiled in Microsoft Excel and analyzed using SPSS version 22.0. Descriptive statistics were expressed as mean and standard deviation. One-way ANOVA followed by Tukey's post hoc test was used to compare differences in percentage inhibition and absorbance values across groups. A *p*-value < 0.05 was considered statistically significant.

Percentage Inhibition of *S. mutans*

Significant differences in inhibition percentages were observed among the three groups at various concentrations.

- Cranberry extract showed significantly higher inhibition at 200 µg, 300 µg, and 400 µg concentrations compared to cinnamon and xylitol.
- Xylitol consistently exhibited the lowest inhibition, particularly at 100 µg, 500 µg, and 600 µg.
- No significant difference was noted between cranberry and cinnamon at 100 µg, 500 µg, and 600 µg.

Table 1 presents the comparative analysis of inhibition percentages across all concentrations.

Absorbance Values

Absorbance values at 620 nm reflect the density of biofilm remaining after treatment:

- Xylitol group exhibited significantly higher absorbance at all concentrations, indicating lower antibiofilm activity.
- Cranberry extract showed lowest absorbance values, especially at 300 µg and 400 µg.
- At 300 µg and 400 µg, cinnamon extract had significantly higher absorbance than cranberry, but no difference was observed at other concentrations.
- All the three samples have exhibited antibacterial activity against s.mutans indicating that they may have a potential for preventing or treating dental caries.

Table 2 summarizes the comparative absorbance values for all three test agents.

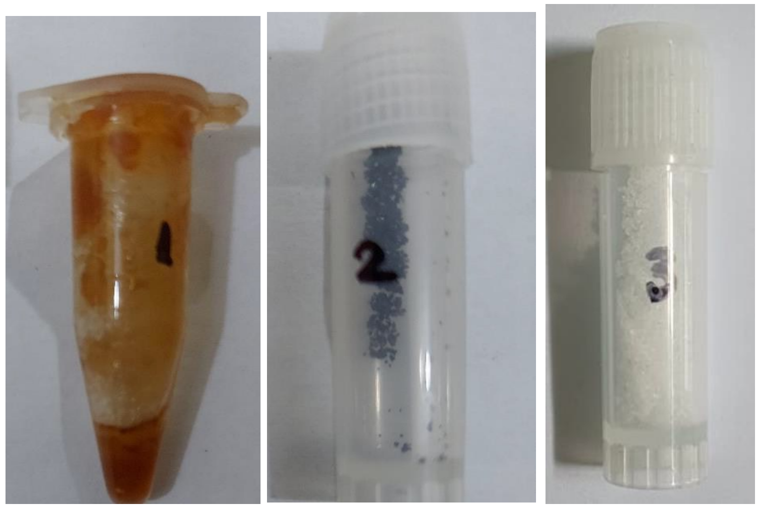

Figure1

Smple 1, Sample 2 and sample 3

Sample 1- Cranberry extract

Sample 2- Cinnamon extract Sample 3- Xylitol

Fig 2: Biofilm formation- *s.mutans*

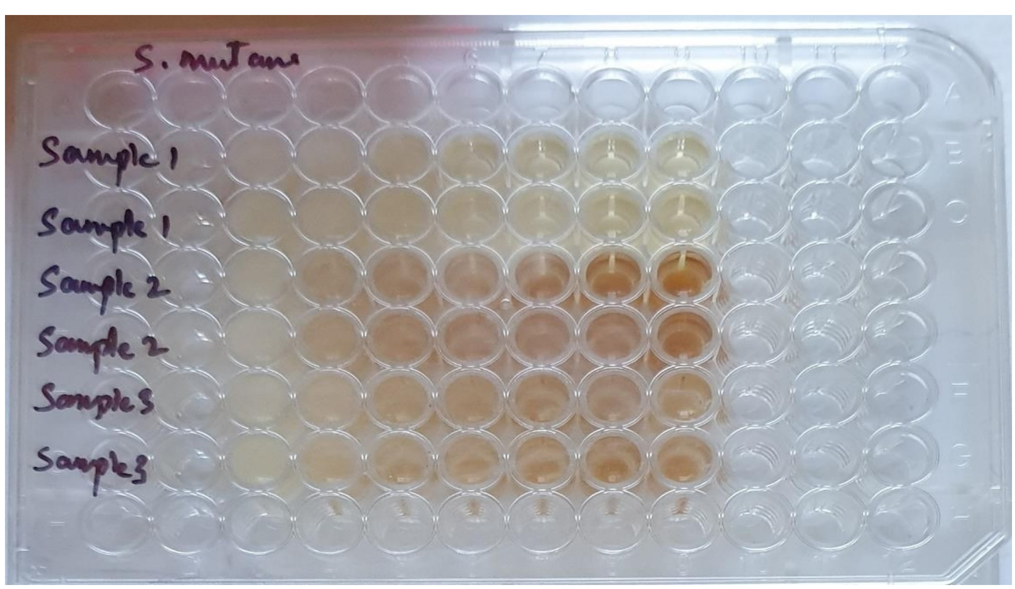

Fig 3: Sample against *s.mutans*

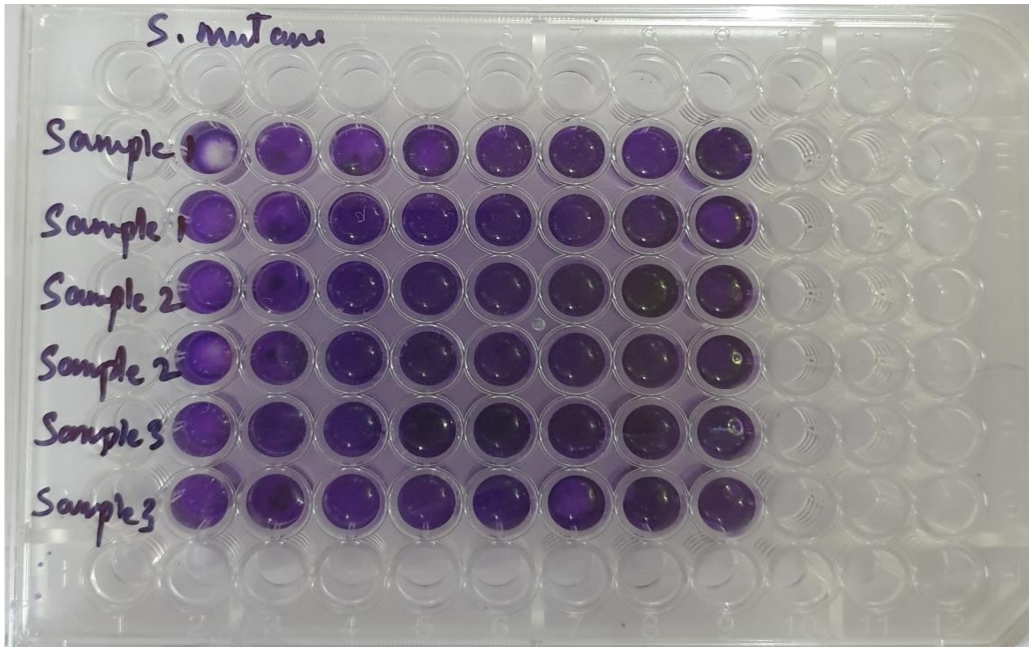

Fig 4: Cystal violet added on *s.mutans* biofilm

Table 1- Comparison of mean percentage of inhibition for S. Mutans b/w diff. groups at various conc. (in □g) using One-way ANOVA test followed by Tukey's Post hoc Test

Conc.	Group 1		Group 2		Group 3		P-Value	Tukey's Post hoc Test		
	Mean	SD	Mean	SD	Mean	SD		G1 vs G2	G1 vs G3	G2 vs G3
Conc.100	8.03	2.33	6.80	0.94	1.31	0.31	0.003*	0.59	0.003*	0.009*
Conc.200	15.87	0.23	9.00	1.47	8.71	4.10	0.02*	0.04*	0.03*	0.99
Conc.300	32.87	3.25	15.73	1.12	12.68	1.68	<0.001*	<0.001*	<0.001*	0.28
Conc.400	49.33	3.88	27.10	0.17	22.45	2.83	<0.001*	<0.001*	<0.001*	0.18
Conc.500	59.80	4.23	52.50	8.03	28.18	3.62	0.001*	0.32	<0.001*	0.004*
Conc.600	64.60	3.42	69.03	5.33	35.79	1.17	<0.001*	0.37	<0.001*	<0.001*

Table 2- Comparison of mean Absorbance values at 620nm for S. Mutans b/w diff. groups at various conc. (in □g) using One-way ANOVA test followed by Tukey's Post hoc Test

Conc.	Group 1		Group 2		Group 3		P-Value	Tukey's Post hoc Test		
	Mean	SD	Mean	SD	Mean	SD		G1 vs G2	G1 vs G3	G2 vs G3
Control	0.2120	0.0066	0.2307	0.0032	0.8327	0.0322	<0.001*	0.50	<0.001*	<0.001*
Conc.100	0.1953	0.0015	0.2157	0.0015	0.8277	0.0287	<0.001*	0.36	<0.001*	<0.001*
Conc.200	0.1783	0.0050	0.2107	0.0021	0.7617	0.0057	<0.001*	<0.001*	<0.001*	<0.001*
Conc.300	0.1417	0.0025	0.1950	0.0053	0.7413	0.0245	<0.001*	0.01*	<0.001*	<0.001*
Conc.400	0.1110	0.0115	0.1690	0.0036	0.6600	0.0505	<0.001*	0.12	<0.001*	<0.001*
Conc.500	0.0843	0.0115	0.1183	0.0203	0.6130	0.0575	<0.001*	0.52	<0.001*	<0.001*
Conc.600	0.0770	0.0104	0.0770	0.0139	0.5427	0.0316	<0.001*	1.00	<0.001*	<0.001*

DISCUSSION

In the present study, all three test agents — cranberry extract, cinnamon extract, and xylitol — exhibited measurable antibacterial activity against *Streptococcus mutans*. However, cranberry extract showed the most significant antibacterial effect, particularly at 200 µg, 300 µg, and 400 µg concentrations, as evidenced by both inhibition percentage and lower absorbance values indicating decreased biofilm formation.

These results are consistent with the study by Yamanaka et al., who demonstrated that cranberry juice significantly inhibits the adhesion of oral streptococci to hydroxyapatite beads by reducing bacterial hydrophobicity, thereby preventing biofilm initiation[9]. The superior performance of cranberry in our study at higher concentrations parallels the findings of Philip and Walsh, who observed that cranberry extract notably reduced metabolic activity and biofilm density of *S. mutans*, suggesting its ability to modulate cariogenic potential without necessarily exerting bactericidal effects[10]. Our findings also align with those of Pellerin et al., who reported that cranberry juice retained its antibacterial effects even after deacidification, supporting its safety and efficacy in maintaining oral health[19].

Cinnamon extract demonstrated moderate antibacterial activity in our study. While its inhibition percentages were lower than cranberry at 300 µg and 400 µg, no statistically significant difference was noted between cranberry and cinnamon at lower and higher concentrations (100 µg, 500 µg, and 600 µg). These findings align with Filoche et al., who reported cinnamon essential oil exhibited potent antimicrobial effects, and even enhanced the efficacy of chlorhexidine against biofilm cultures[11]. Rashad's study further supports our findings, as different concentrations of water cinnamon extract were shown to significantly reduce *S. mutans* counts, with effects comparable to commercial mouthwashes[12]. Similarly, Wiwattanarattanabut et al. demonstrated that cinnamon essential oil was effective against *S. mutans* and had good anti-plaque activity[13].

The absorbance values in our study indicated that cinnamon was less effective than cranberry in disrupting established biofilms at 300 µg and 400 µg, which may be due to differences in phytochemical composition or bioavailability. Nevertheless, cinnamon's capacity to suppress biofilm formation was supported by Alshahrani and Gregory, who observed that cinnamon extract significantly inhibited nicotine-induced biofilm formation by *S. mutans*, indicating strong antibiofilm potential even under stress conditions[17]. Gupta et al. also confirmed cinnamon oil's superior antimicrobial activity over clove oil and chlorhexidine, further validating our in vitro findings[18]. Furthermore, Sherief et al. noted enhanced antibacterial effects when cinnamon was incorporated into restorative materials, suggesting that it can enhance both therapeutic and preventive dental care strategies[20].

Xylitol demonstrated the least antibacterial activity in our study. It showed the lowest inhibition percentages and the highest absorbance values at all concentrations, indicating reduced effectiveness against *S. mutans* and its biofilms under in-vitro conditions. However, previous studies have emphasized its unique mechanism of action. For instance, Kayalvizhi et al. demonstrated a significant reduction in *S. mutans* counts among young children using xylitol wipes, likely due to interference with bacterial metabolism and not direct bacterial killing[15]. Wang et al. also reported that daily xylitol wipe use was effective in controlling plaque, although their sample size was small[16]. While our in-vitro results suggest limited biofilm disruption by xylitol, the systematic review by Söderling et al. highlighted xylitol's ability to reduce plaque accumulation in clinical settings, supporting its role in preventive care[22].

Additionally, the moderate activity of xylitol in our study mirrors the findings of Hajiahmadi et al., who observed that xylitol gels had modest antibacterial effects compared to propolis and aloe vera[21]. Interestingly, Galganny-Almeida found that xylitol-based tooth wipes provided effective plaque control and were well-accepted by parents of high-caries-risk infants, suggesting xylitol's practicality in pediatric populations despite its limited antibacterial strength in vitro[14].

Taken together, our findings suggest that cranberry extract holds the greatest promise as an antibacterial and antibiofilm agent against *S. mutans*. Cinnamon extract also demonstrated considerable efficacy and could serve as an alternative natural agent. Although xylitol was least effective in our in-vitro setup, its long-term benefits in clinical caries prevention — particularly in children — make it a valuable adjunct in oral hygiene regimens.

In the present in-vitro study, cranberry extract demonstrated the highest antibacterial efficacy against *Streptococcus mutans*, particularly at concentrations of 200 µg, 300 µg, and 400 µg. This was evident in both the percentage inhibition and reduced absorbance values, suggesting potent antibiofilm activity. These findings are strongly supported by Yamanaka et al., who reported that cranberry juice reduced bacterial adhesion to saliva-coated hydroxyapatite by decreasing bacterial hydrophobicity, indicating its ability to prevent early colonization and biofilm formation[9]. Furthermore, Philip and Walsh observed that cranberry extracts significantly reduced metabolic activity, acid production, and exopolysaccharide volume in *S. mutans* biofilms, supporting our observation of cranberry's superior inhibitory potential[10].

Cinnamon extract showed moderate antibacterial activity in our study. While it was not as effective as cranberry at mid-range concentrations (300 µg and 400 µg), it showed comparable efficacy at lower and higher concentrations. This aligns with Filoche et al., who reported strong antimicrobial potency of cinnamon essential oil and its synergistic action with chlorhexidine against biofilms[11]. Similarly, Rashad demonstrated that water cinnamon extract significantly reduced *S. mutans* counts in vitro and in vivo, reinforcing its potential as a natural antibacterial agent[12]. Wiwattanarattanabut et al. also identified cinnamon as one of the most effective herbal extracts with anti-cariogenic activity against *S. mutans* and *Lactobacillus casei*[13].

Xylitol demonstrated the lowest antibacterial activity among the three test agents in our study. This was evident in the consistently higher absorbance values across all concentrations, indicating weaker biofilm disruption. However, it is important to note that xylitol's anticaries effect is not primarily bactericidal but instead interferes with bacterial metabolism and adhesion. Galganny-Almeida showed that xylitol-based tooth wipes significantly reduced plaque levels and had higher parental acceptance, indicating clinical relevance despite its modest in-vitro activity[14]. Kayalvizhi et al. observed significant reduction in *S. mutans* counts in children using xylitol wipes, supporting its role in caries prevention in pediatric settings[15]. Wang et al. also confirmed its plaque-reducing effect in young children, though their study emphasized the need for larger sample sizes to validate the findings[16].

The findings for cinnamon in our study are further supported by Alshahrani and Gregory, who observed that cinnamon water extract significantly inhibited nicotine-induced *S. mutans* biofilm formation, indicating its strong antibiofilm activity even under environmental stress[17]. Gupta et al. reported that cinnamon oil had broad-spectrum antimicrobial activity, performing better than clove oil and chlorhexidine against caries-causing organisms[18]. Pellerin et al. also demonstrated that deacidified cranberry juice retained its antimicrobial effects against *S. mutans* and *S. sobrinus*, and improved oral epithelial barrier function, aligning with our study's results on cranberry[19].

Sherief et al. investigated the incorporation of cinnamon oil into glass ionomer cement and found improved antimicrobial efficacy against *S. mutans* and *C. albicans*, with enhanced fluoride release and compressive strength[20]. Similarly, Hajiahmadi et al. compared several gels and found xylitol to exhibit moderate antibacterial effects — consistent with the limited efficacy seen in our in-vitro results[21].

Collectively, these findings suggest that cranberry extract possesses the most promising antibacterial and antibiofilm properties among the three agents tested. Cinnamon also exhibited considerable efficacy and may serve as a natural alternative to conventional agents. Xylitol, although less effective in vitro, continues to be a valuable preventive agent, particularly in pediatric and community dental care due to its safety and plaque-inhibiting properties.

CONCLUSION

Within the limitations of this in-vitro study, it can be concluded that cranberry extract exhibited the highest antibacterial and antibiofilm activity against *Streptococcus mutans*, followed by cinnamon extract, while xylitol showed the least inhibitory effect. The findings suggest that cranberry and cinnamon extracts possess promising potential as natural antimicrobial agents for preventing or reducing dental caries. Although xylitol demonstrated minimal in-vitro antibacterial activity, its established clinical benefits warrant continued use as a supportive oral hygiene agent. Further clinical studies are needed to validate these findings and explore formulation-based applications for effective caries prevention.

Key Message

Cranberry and cinnamon extracts demonstrated significant antibacterial activity against *Streptococcus mutans*, highlighting their potential as natural alternatives in caries prevention. Cranberry extract, in particular, showed superior inhibition and biofilm disruption, whereas xylitol exhibited the least effect in vitro. These findings support the incorporation of plant-based agents in preventive oral care formulations.

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