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Antimicrobial Activity Of Prunus Cerasus L Mouthwash - An In Vitro Microbial Study

Research Article

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Abstract

Introduction: Mutans streptococci are the main cause of tooth decay. Application of natural materials as mouthwash has been effective in reducing the bacterial count. This study aimed to assess the antimicrobial effects of Sour cherry mouthwash on two oral bacteria S. mutans and S. sobrinus responsible for tooth decay.
Materials and Methods: Strongly adherent strains of Streptococcus mutans and Streptococcus sobrinus were selected for this in vitro study. Antimicrobial effects of Sour cherry and Pomegranate mouthwash on microbial count in the biofilm and adhesion potential of bacteria were evaluated by microtiter plate method. Also, the well-plate technique was used to assess the effect of Sour cherry and Pomegranate mouthwash in comparison with CHX on bacterial growth and proliferation. The obtained results were analyzed by one-way ANOVA and Tukey's post hoc test using SPSS version 20.0 with Level of significance was set at P=0.05 with 95% confidence interval.
Results: The chlorhexidine (CHX), Sour cherry and Pomegranate mouthwashes decreased plaque formation by S. mutans by 93%, 80% and 68%, respectively. These values for the S. sobrinus were 92%, 57% and 48%, respectively (P<0.001). CHX was more effective than the other two materials (P<0.001). However, none of these materials eliminated the biofilm. Sour cherry and Pomegranate mouthwash inhibited the growth of the afore-mentioned bacteria.
Conclusion: Within the limitations of this study, it showed that Sour cherry and Pomegranate mouthwash have the potential to prevent or control the proliferation of S. mutans and S. sobrinus.

Keywords: Dental Caries; Mouthwashes; Streptococcus Mutans; Streptococcus Sobrinus.

Introduction

Expanded open interest for natural products in treatment of diseases has resulted in a search for herbal medications. There are approximately 500,000 plant species worldwide; out of which, only 1% have been photo-chemically evaluated and there is a great potential for finding bioactive materials [1]. Several articles are available on the antimicrobial efficacy of plant extracts; however, the effects of medicinal plants on oral pathogenic microorganisms have been less commonly evaluated.

Dental caries is a common infectious disease with higher prevalence among lower socioeconomic groups [1]. Treatment of caries is time consuming and costly. Considering the high prevalence of caries, increased bacterial resistance to antibiotics, adverse effects of chemical and synthetic drugs as well as their high cost, there is a clear need for natural, and cost effective alternatives for prevention of caries. The currently available chemical medications change the oral microbiota and have complications such as diarrhea, vomiting and tooth staining [2, 3]. Streptococcus mutans and S. sobrinus are the main causes of caries due to their high potential for biofilm formation. These bacteria adhere to tooth enamel and produce glucans following the consumption of sucrose in foods and result in accumulation of glucans in the biofilm and eventually cause enamel destruction via acid production.

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[4] Since bacterial adhesion is the first step for bacterial proliferation and tooth decay, adhesion is the new target for the modalities aiming to inhibit bacterial colonization.[3] In this consideration, medicinal plants have also been evaluated. Studies in this regard are divided into two groups. Group one, studies assess the activity of natural compounds, plant extracts and pure photochemical agents against oral pathogenic microorganisms and group two, studies assess the efficacy of these products for inhibition of oral biofilm formation via decreasing microbial attachment to tooth surfaces.[5] Materials that have a potential to inhibit bacterial adhesion and growth would be ideal for prevention of tooth caries. Among the herbal alternatives, sour cherry finds a special mention. Sour cherry (botanical name: Prunus cerasus) is one of the oldest known edible fruits. They are also known by other names such as sour, tart, dwarf or wild cherry, a traditional Unani medicine, popularly known as Aloo baloo.[6] It has been widely used in traditional medicine for the treatment of different types of diseases around the world. Furthermore, it has been reported that the antioxidant capacity ranges from 1,145 to 1,916 µmol TE/100 grams in sour cherries.[7-9]

Previously our team has a rich experience in working on various research projects across multiple disciplines [10-24] Now the growing trend in this area motivated us to pursue this project. This study aimed to assess the antimicrobial effect of Pomegranate and sour cherry mouthwash on oral bacteria. Since the antimicrobial effects of these two substances on oral streptococci have not been evaluated.

Materials And Methods

In this in vitro study, five strains of S. mutans were isolated from dental caries and dental plaque of patients using a sterile curette. Standard strains of S. mutans (ATCC35668) and S. sobrinus (ATCC27607) were also obtained. A suspension was prepared of the lyophilized powder. All strains were cultured in blood agar and Mitis Salivarius agar (MSA) and incubated at 37°C and 5% CO2 for 24 hours. Standard strains were cultured, exposed to bacitracin and optochin discs, gram stained and subjected to catalase test for final confirmation.

Determining the quantity of biofilm formation by the bacteria using microtiter plate method

The microtiter plate method is based on colorimetry and is used to determine the biofilm formation potential of bacteria and assessment of the effect of antimicrobial agents on the biofilm. This method requires a small culture medium, it is not time consuming and can be used for assessment of the antimicrobial efficacy of a wide range of antimicrobial agents with different concentrations and in combination with each other.[25, 26]

In order to assess the biofilm formation potential of the bacteria, 18-24 hour culture of the bacteria in Tryptic Soy Broth (TSB) supplemented with 1% sucrose and 5% CO_2 was prepared. This microbial suspension was diluted with sterile TSB to obtain 0.5 McFarland turbidity. Of this suspension, 250µL was transferred to eight wells of a polystyrene 96-well plate. The control wells only contained sterile culture medium. After 24 hours, the content of the wells was removed and each well was rinsed with 300µL of sterile saline. Next, bacteria attached to the walls and bottom of the wells were fixed with 250µL of 96% ethanol. After 15 minutes, the contents of the wells were removed and the plates were dried at room temperature. Then, they were stained with 2% crystal violet for five minutes and after washing the excess dye with water, the plates were dried at room temperature. Next, 200 µL of 33% glycol acetic acid was added to each well and the optical density (OD) of the crystal violet present in the solvent was read by an ELISA reader at 492nm wavelength. Bacterial strains were then classified based on their OD as follows:[27]

OD: The mean light absorbance of bacteria
ODc: The mean light absorbance of control wells
ODIODc: No adhesion
ODc<ODI2OD: Poor adhesion
2ODc<ODI4ODc: Moderate adhesion
4ODc<OD: Strong adhesion

Strains with strong adhesion, which had greater biofilm formation potential than others were chosen for the next step. Determination of the efficacy of Pomegranate , Sour cherry and CHX mouthwash for elimination of biofilm: Microtiter-plate method was used for this purpose. After preparation of 18-20 hour culture, 0.5 McFarland standard suspensions were prepared of the strains with strong adhesion in TSB supplemented with 1% sucrose. The suspensions were then diluted 1/100 in sterile TSB and all wells of a polystyrene 96-well plate were filled with 250µL of this suspension. Control wells only contained aqueous medium. After inoculation, the plates were covered and incubated at 37°C and 5% CO2 for 24 hours. Then, the wells were emptied and washed with 300µL of sterile saline; 250µL of Sour cherry, Pomegranate and CHX mouthwash were added using 0.2 um Millipore syringe filter for one hour.

All eight wells in each row were treated the same and the antimicrobial agents were refreshed every 20 minutes. The control well rows only contained biofilm. After one hour, antimicrobial agents were removed by washing the wells. Next, the wells were stained with 200µL of 2% crystal violet for five minutes and after rinsing, they were filled with 200µL of 33% glycol acetic acid. In the next step, they were shaken on a shaker for 15 minutes and their OD was read at 492nm wavelength by an ELISA reader. Assessment of the efficacy of these materials was done by calculating the percentage of reduction in biofilm via OD of treated and control wells using the formula below:[28]

Percentage of biofilm reduction = (C-B)-(T-B) × 100/(C-B)

Where C is the mean OD of positive control wells, B is the mean OD of negative control wells and T is the mean OD of treated wells.

Overnight culture of strongly adherent streptococci in TSB medium supplemented with 1% sucrose was done. To assess the effect of these materials on bacterial adhesion, two methods were used: In the first method, 200µL of a mixture with similar portions of antimicrobial agent and bacteria was transferred to the wells while the control wells only contained microbial suspension. In the second method, 100µL of Pomegranate, Sour cherry and CHX mouthwash were poured into wells and streptococcal suspension was added 30 minutes later. In the control wells, first 100µL of phosphate buffered saline (PBS) and after 30 minutes, 100µL of streptococcal suspension were added. After 24 hours of incuba-

tion, the solutions and nutrients were removed from the wells and after three times of rinsing with PBS, staining was performed using 2% crystal violet for five minutes. After addition of 33% acetic acid, OD of the solvent was read using an ELISA reader.

The well-plate technique was used to assess the effect of antibacterial agents in this respect [29]. After 18-20 hour culture of S. mutans and S. sobrinus in brain heart infusion broth, a microbial suspension with 0.5 McFarland standard concentration was prepared of the bacteria and cultured on Mueller Hinton agar supplemented with 5% defibrinated sheep blood using a sterile swab in three directions with a 60° angle. Inoculated plates were placed on a smooth surface for three to five minutes and then, equal wells measuring 6 mm in diameter were created in the medium using the tip of a sterile pasteur pipette. One drop of melted Mueller Hinton agar was poured into each well in order to seal the bottom of the wells. Next, 100 µL of the antimicrobial agents were added to each well.

Distilled water was used as the negative control. The plates were refrigerated for one hour in order for the antimicrobial agents to be able to diffuse in the medium before bacterial proliferation. The plates were incubated for 16-20 hours and the diameter of the growth inhibition zone was then measured.

Statistical Analysis

The data were analyzed using SPSS version 20.0. One-way ANOVA and Tukey's post hoc test were used for analysis of the data related to S. mutans, and the Kruskal Wallis test and Mann Whitney U test were used to analyze the data regarding S. sobrinus. Level of significance was set at P=0.05.

Results

Assessment of the Quantity of Biofilm Formation by Streptococci

To assess the effect of Sour cherry, Pomegranate and CHX on bacterial adhesion, first the quantity of formed biofilm was assessed in order to select strains with greater adhesion to assess the effect of these antimicrobial agents on adhesion of bacteria with strong adhesion. (Figure 1) shows the mean of three measurements of OD of bacteria. Standard S. mutans and S. sobrinus strains (bacteria #1 and 2) were the strongly adherent strains. Bacteria # 4-7 isolated from the mouth of candidates had poor adhesion and bacterium #3 was non-adherent.

Effect of Understudy Materials on S. Mutans and S. Sobrinus Biofilm

The results showed that Pomegranate, Sour Cherry and CHX were able to prevent biofilm formation by these bacteria. The percentage of reduction in biofilm formation in presence of Sour cherry, Pomegranate, CHX was 0.089%, 0.38% and 0.089%, respectively for S. mutants and 0.061%, 0.37% and 0.062%, respectively for S. sobrinus; no statistically significant difference was noted in this respect (P=0.514).

Effect of Understudy Materials on Strongly Adherent Bacteria

The effects of pomegranate, Sour cherry, CHX on adhesion of S. mutans and S.sobrinus are presented in (Table 1). All three materi-

Figure 1. Bar chart showing the association between the mean diameter of growth inhibition of s.mutans and s.sobrinus against three different mouthwashes, X axis represents the bacteria, Y axis represents the diameter of growth inhibition; sour cherry mouthwash (gren) is better than pomegranate mouthwash (blue) and statistically significant when compared with each other.(F=49.542) and p value 0.003).

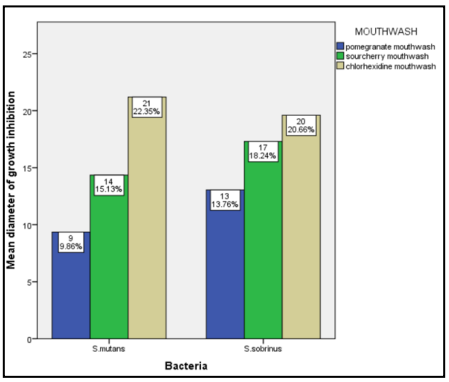


Table 1. The percentage of reduction in adhesion of S. Mutans and S. Sobrinus in presence of pomegranate, Sour cherry and CHX mouthwash.

Material	Bacteria	Percentage of reduction in adhesion(in method 1)	Standard deviation	Percentage of reduction in adhesion (in method 2)	Standard deviation
Pomegranate Mouthwash	S. mutans	80	0.06	56	0.08
	S. sobrinus	57	0.1	60	0.16
Sour cherry Mouthwash	S. mutans	93	0.01	95	0.02
	S. sobrinus	92	0.03	93	0.03
CHX	S. mutans	68	0.11	52	0.06
	S. sobrinus	48	0.1	32	0.12

als significantly decreased the adhesion of S. mutans and S. sobrinus compared to the control group (P<0.001). As seen in Table 1, Pomegranate resulted in 80 and 56% reduction in adhesion of S. mutans (in the first and second methods, respectively) and 57% and 60%, respectively for the adhesion of S. sobrinus, which indicate no significant difference in the two methods for the effect of sour cherry on S. sobrinus (P=0.451). Also, the effect of sour cherry on adhesion of S. mutans in the second method had no significant difference with that of CHX (P=0.319). However, the second method was effective for S. sobrinus (P=0.003). Sour cherry inhibited the adhesion of both bacteria and had the greatest effect on bacterial adhesion compared to other materials in both methods (P<0.001); no significant difference was observed between the two methods in this respect (P=0.514) CHX significantly decreased bacterial adhesion compared to the control group (P<0.001). No significant difference was found between the two methods for S. sobrinus, and both methods decreased the adhesion of both bacteria to the same extent (P=0.105).

Discussion

Our institution is passionate about high quality evidence based research and has excelled in various fields [30-40].

Several studies have assessed the antimicrobial efficacy of herbal mouthwash for reduction of dental plaque. This mouthwash has antimicrobial effects on oral pathogenic bacteria [41-43]. The current study showed the efficacy of Pomegranate in decreasing the adhesion of oral streptococci; this result is in agreement with that of other studies on the effect of this mouthwash on oral bacteria. On the other hand, inability of Pomegranate to eliminate biofilm or inhibit the growth and proliferation of oral bacteria was noted as well. Brazilian researchers evaluated the effect of Pomegranate extract on adhesion of oral bacteria (which is the first step in development of caries).[44]

Various studies have already shown that sour cherry has various

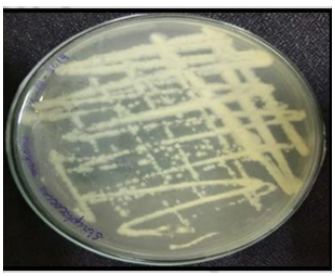
Table 2. The mean diameter of growth inhibition zones for S.Mutans and S.Sobrinus in presence of pomegranate mouthwash, sour cherry mouthwash and chlorhexidine mouthwash.

Material	Bacteria	Mean diameter of growth inhibition zone in well-plate method(mm)	Standard deviation
Pomegranate Mouthwash	S. mutans	9	0.79
	S. sobrinus	13	1
Sour Cherry Mouthwash	S. mutans	14	0.89
	S. sobrinus	17	0.95
CHX	S. mutans	21	1.71
	S. sobrinus	20	1.5

Figure 2. Sour Cherry Fruit Used.

Figure 3. Sour Cherry Mouthwash Preparation.

Figure 4. Colony Formation on the culture media.



active components. Sour cherries contain higher total phenolics than sweet cherries due to higher anthocyanin and hydroxycinnamic acid concentrations, consisted primarily of cyanidin 3-O-rutinoside, cyanidin 3-O-glucoside and minor amounts of delphinidin, malvidin, peonidin, petunidin glycosides.[45, 46]. When used regularly in combination with toothpaste that has been reinforced with bioactive botanical extracts, sour cherries containing mouthwash may fight dental plaque and tartar formation by inhibiting the activities of the microorganisms that cause plaque. [47, 48] In addition, sour cherry compounds possess anti-inflammatory properties that may help soothe irritated tissues [49-51] Its extract suppresses the ability of these microorganisms to adhere to the surface of the tooth. Plaque may involve four or more different microorganisms combining forces to colonize the surface of the teeth.[47, 48] Remarkably, nature's own sour cherry fights the organism's ability to adhere by interfering with the production of the very chemicals the bacteria use for adhesion.[47] In a study done by J R Homoki et al., 2018, subjects were given chewing gum with and without sour cherry extract and experienced a reduction in salivary S.mutans levels which is normally higher among people with caries and may correlate with plaque forming bacterial content [52]. Sour cherry rinsing also increased saliva activities of alpha amylase, an enzyme that helps in clearance of streptococcus mutans and performs a direct inhibitory effect on the growth of certain bacteria or viruses [53-55]. Therefore, sour cherries may exhibit anticariogenic effects as well, which may be utilized to prevent dental caries in individuals.

A few studies are available on the antimicrobial effects of compounds derived from Pomegranate on oral pathogenic microorganisms. Loo et al, in 2010 reported the effect of lactic acid on oral bacteria [56]. Pomegranate extract contains about 40% lactic acid. Also, Vasconcelos et al., in 2006 showed the antimicrobial effects of Pomegranate extract on S. mutans [44]. Subramaniam et al., 2012 compared the antimicrobial effects of Pomegranate extract with those of Aloe Vera and sorbitol and showed that Pomegranate extract had greater antimicrobial efficacy compared to the other two against S. mutans [57].

The current results showed that Sour cherry significantly inhibited the adhesion and proliferation of bacteria; however, after biofilm formation, it could not eliminate it. Sour cherry may be suitable for elimination of oral bacteria since it can inhibit adhesion and proliferation of bacteria at the same time. Search of the literature yielded no study on the effect of Sour cherries on plaque producing oral bacteria. Thus, the current study is the first to assess the effect of Sour cherry mouthwash on oral bacteria. The results showed that Sour cherry mouthwash had a significant effect on decreasing the adhesion of bacteria but had no effect on their growth and could not eliminate biofilm after its formation. This finding shows that the mechanism of Sour cherry in decreasing biofilm formation is different from its bactericidal and growth inhibition mechanisms; however, a definite conclusion in this regard requires further studies. The results showed that Pomegranate, Sour cherry and CHX were all effective against adhesion of oral bacteria; however, they were not effective for elimination of biofilm. This finding may be due to strong adhesion of dental biofilm due to the activity of mutans streptococci. They first attach to plates by sucrose-independent adsorption and then sucrose-dependent adherence occurs following the synthesis of insoluble glucans. Therefore, these colonization mechanisms may be so intense that they prevent the effect of antimicrobial agents on elimination of biofilm.Future studies are required to further assess the effects of Sour cherry in effective formulations on dental caries in clinical trials.

Clinical Significance

Sour cherry mouthwash can be used as an effective adjunctive with dentifrices for reduction of oral bacteria which causes dental caries. The antioxidant effect of the sour cherry will be an advantage for oral tissues.

Conclusion

In this study it was proven that the optimal antimicrobial effects of Sour cherry on proliferation of cariogenic streptococci and the optimal efficacy of Pomegranate and Sour cherry in decreasing the adhesion of these bacteria, future studies are recommended to assess the application of these materials in different pharmaceutical formulations for caries prevention.

Data Availability

The experimental data used to support the findings of this study are included within the article.

Conflicts Of Interest

All authors declare that they have no conflicts of interest. In addition, all authors have read and approved the manuscript as submitted, are qualified for authorship, believe the submission represents honest work and take full responsibility for the reported findings.

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References

[1]. Curzon MEJ, Preston AJ (2004) Risk Groups: Nursing Bottle Caries/Caries in the Elderly. Caries Research 38:24–33
[2]. Palombo EA (2011) Traditional Medicinal Plant Extracts and Natural Products with Activity against Oral Bacteria: Potential Application in the Prevention and Treatment of Oral Diseases. Evidence-Based Complementary and Alternative Medicine 2011:1–15
[3]. Lamont RJ, Jenkinson HF (2010) Oral Microbiology at a Glance. John Wiley & Sons
[4]. Taubman MA, Nash DA (2006) The scientific and public-health imperative for a vaccine against dental caries. Nature Reviews Immunology 6:555–563
[5]. Steinberg D (2004) Effect of a high-molecular-weight component of cranberry on constituents of dental biofilm. Journal of Antimicrobial Chemotherapy 54:86–89
[6]. Elwes HJ, Henry A PRUNUS CERASUS, DWARF CHERRY. The Trees of Great Britain and Ireland 1541–1542
[7]. Blando F, Oomah BD (2019) Sweet and sour cherries: Origin, distribution, nutritional composition and health benefits. Trends Food Sci Technol 86:517–529
[8]. Hosseini S, Parastouei K, Khodaiyan F (2020) Simultaneous extraction optimization and characterization of pectin and phenolics from sour cherry pomace. Int J Biol Macromol 158:911–921
[9]. Shamsi S (2017) A review on sour cherry (Prunus cerasus): A high value Unani medicinal fruit. International Journal of Green Pharmacy (IJGP) 11:

[10]. Govindaraju L, Gurunathan D (2017) Effectiveness of Chewable Tooth Brush in Children-A Prospective Clinical Study. J Clin Diagn Res 11:ZC31–ZC34
[11]. Christabel A, Anantanarayanan P, Subash P, Soh CL, Ramanathan M, Muthusekhar MR, Narayanan V (2016) Comparison of pterygomaxillary dysjunction with tuberosity separation in isolated Le Fort I osteotomies: a prospective, multi-centre, triple-blind, randomized controlled trial. Int J Oral Maxillofac Surg 45:180–185
[12]. Soh CL, Narayanan V (2013) Quality of life assessment in patients with dentofacial deformity undergoing orthognathic surgery--a systematic review. Int J Oral Maxillofac Surg 42:974–980
[13]. Mehta M, Deeksha, Tewari D, et al (2019) Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. Chem Biol Interact 308:206–215
[14]. Ezhilarasan D, Apoorva VS, Ashok Vardhan N (2019) Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med 48:115–121
[15]. Campeau PM, Kasperaviciute D, Lu JT, et al (2014) The genetic basis of DOORS syndrome: an exome-sequencing study. Lancet Neurol 13:44–58
[16]. Kumar S, S S (2016) Knowledge and awareness regarding antibiotic prophylaxis for infective endocarditis among undergraduate dental students. Asian J Pharm Clin Res 154
[17]. Christabel SL (2015) Prevalence of type of Frenal Attachment and morphology of frenum in children, Chennai, Tamil Nadu. World J Dent 6:203–207
[18]. Kumar S, Rahman R (2017) Knowledge, awareness, and practices regarding biomedical waste management among undergraduate dental students. Asian J Pharm Clin Res 10:341
[19]. Sridharan G, Ramani P, Patankar S (2017) Serum metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Cancer Res Ther 13:556–561
[20]. Ramesh A, Varghese SS, Doraiswamy JN, Malaiappan S (2016) Herbs as an antioxidant arsenal for periodontal diseases. J Intercult Ethnopharmacol 5:92–96
[21]. Thamaraiselvan M, Elavarasu S, Thangakumaran S, Gadagi JS, Arthie T (2015) Comparative clinical evaluation of coronally advanced flap with or without platelet rich fibrin membrane in the treatment of isolated gingival recession. J Indian Soc Periodontol 19:66–71
[22]. Thangaraj SV, Shyamsundar V, Krishnamurthy A, Ramani P, Ganesan K, Muthuswami M, Ramshankar V (2016) Molecular Portrait of Oral Tongue Squamous Cell Carcinoma Shown by Integrative Meta-Analysis of Expression Profiles with Validations. PLoS One 11:e0156582
[23]. Ponnulakshmi R, Shyamaladevi B, Vijayalakshmi P, Selvaraj J (2019) In silico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats. Toxicol Mech Methods 29:276–290
[24]. (2018) Fluoride, fluoridated toothpaste efficacy and its safety in children - review. Int J Pharm Res.
[25]. Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M (2000) A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods 40:175–179
[26]. Pitts B, Hamilton MA, Zelver N, Stewart PS (2003) A microtiter-plate screening method for biofilm disinfection and removal. Journal of Microbiological Methods 54:269–276
[27]. O'Toole GA, Kolter R (1998) Initiation of biofilm formation inPseudomonas fluorescensWCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. Molecular Microbiology 28:449–461
[28]. Sonak S, Bhosle NB (1995) A simple method to assess bacterial attachment to surfaces. Biofouling 9:31–38
[29]. Yassa N, Tofighi Z, Molazem M, Aliaslmamghany F, Shahverdi A, Samadi N (2011) Evaluation of antimicrobial effects of three traditional medicinal plants from Iran. Planta Medica.
[30]. Vijayashree Priyadharsini J (2019) In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol 90:1441–1448
[31]. Pc J, Marimuthu T, Devadoss P (2018) Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study. Clin. Implant Dent. Relat. Res.
[32]. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S (2018) Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. J Periodontol 89:1241–1248
[33]. Ramadurai N, Gurunathan D, Samuel AV, Subramanian E, Rodrigues SJL (2019) Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial. Clin Oral Investig 23:3543–3550
[34]. Sridharan G, Ramani P, Patankar S, Vijayaraghavan R (2019) Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma. J Oral Pathol Med 48:299–306
[35]. Ezhilarasan D, Apoorva VS, Ashok Vardhan N (2019) Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med 48:115–121
[36]. Mathew MG, Samuel SR, Soni AJ, Roopa KB (2020) Evaluation of adhesion of Streptococcus mutans, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: Randomized controlled trial. Clin Oral Investig 1–6
[37]. Samuel SR (2021) Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent 31:285–286
[38]. R H, Hannah R, Ramani P, Ramanathan A, R JM, Gheena S, Ramasubramanian A, Monika K (2020) CYP2 C9 polymorphism among patients with oral squamous cell carcinoma and its role in altering the metabolism of benzo[a]pyrene. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology 130:306–312
[39]. Chandrasekar R, Chandrasekhar S, Sundari KKS, Ravi P (2020) Development and validation of a formula for objective assessment of cervical vertebral bone age. Prog Orthod 21:38
[40]. Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A (2018) In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species. Arch Oral Biol 94:93–98
[41]. Al-Bayati FA, Sulaiman KD (2008) In vitro antimicrobial activity of Salvadora persica L. extracts against some isolated oral pathogens in Iraq. Turk J Biol 32:57–62
[42]. Mohanty S, Ramesh S (2017) Antimicrobial efficacy of apple cider vinegar against Enterococcus faecalis and Candida albicans: An in vitro study. Journal of Advanced
[43]. Khirtika SG, Ramesh S (2017) Comparative Evaluation of Antimicrobial efficacy of 0.2% Chlorhexidine, 2% Iodine and Homemade Mouthrinse as an Anti-Caries Agent-A Clinical Study. Sciences and Research
[44]. Vasconcelos LC de S, Sampaio FC, Sampaio MCC, Pereira M do SV, Higino JS, Peixoto MHP (2006) Minimum inhibitory concentration of adherence of Punica granatum Linn (pomegranate) gel against S. mutans, S. mitis and C. albicans. Braz Dent J 17:223–227
[45]. Średnicka-Tober D, Ponder A, Hallmann E, Głowacka A, Rozpara E (2019) The Profile and Content of Polyphenols and Carotenoids in Local and Commercial Sweet Cherry Fruits (L.) and Their Antioxidant Activity In Vitro. Antioxidants (Basel).
[46]. Özen M, Özdemir N, Ertekin Filiz B, Budak NH, Kök-Taş T (2020) Sour cherry (Prunus cerasus L.) vinegars produced from fresh fruit or juice concentrate: Bioactive compounds, volatile aroma compounds and antioxidant capacities. Food Chem 309:125664
[47]. Ben Lagha A, LeBel G, Grenier D (2020) Tart cherry (Prunus cerasus L.) fractions inhibit biofilm formation and adherence properties of oral pathogens and enhance oral epithelial barrier function. Phytother Res 34:886–895
[48]. Homoki JR, Nemes A, Fazekas E, et al (2016) Anthocyanin composition, antioxidant efficiency, and α-amylase inhibitor activity of different Hungarian sour cherry varieties (Prunus cerasus L.). Food Chem 194:222–229
[49]. Biro A, Markovich A, Homoki JR, Szőllősi E, Hegedűs C, Tarapcsák S, Lukács J, Stündl L, Remenyik J (2019) Anthocyanin-Rich Sour Cherry Extract Attenuates the Lipopolysaccharide-Induced Endothelial Inflammatory Response. Molecules.
[50]. Yılmaz FM, Görgüç A, Karaaslan M, Vardin H, Ersus Bilek S, Uygun Ö, Bircan C (2019) Sour Cherry By-products: Compositions, Functional Properties and Recovery Potentials - A Review. Crit Rev Food Sci Nutr 59:3549–3563
[51]. Šarić A, Sobočanec S, Balog T, Kušić B, Šverko V, Dragović-Uzelac V, Levaj B, Čosić Z, Šafranko ŽM, Marotti T (2009) Improved Antioxidant and Anti-inflammatory Potential in Mice Consuming Sour Cherry Juice (Prunus Cerasus cv. Maraska). Plant Foods for Human Nutrition 64:231–237
[52]. Homoki J, Gyémánt G, Balogh P, et al (2018) Sour cherry extract inhibits human salivary α-amylase and growth ofStreptococcus mutans(a pilot clinical study). Food & Function 9:4008–4016
[53]. Scannapieco FA, Torres G, Levine MJ (1993) Salivary α-Amylase: Role in Dental Plaque and Caries Formation. Critical Reviews in Oral Biology & Medicine 4:301–307
[54]. Ochiai A, Harada K, Hashimoto K, Shibata K, Ishiyama Y, Mitsui T, Tanaka T, Taniguchi M (2014) α-Amylase is a potential growth inhibitor ofPorphyromonas gingivalis, a periodontal pathogenic bacterium. Journal of Periodontal Research 49:62–68
[55]. Sato K, Tokuhisa S, Inaba Y (1995) Effect of Enzymes on the Growth of Human and Animal Rotaviruses. The Journal of Veterinary Medical Science 57:569–570
[56]. Loo WTY, Jin LJ, Cheung MNB, Chow LWC (2010) Evaluation of Ellagic acid on the activities of oral bacteria with the use of adenosine triphosphate (ATP) bioluminescence assay. Afr J Biotechnol 9:3938–3943
[57]. Subramaniam P, Uma E, Dwivedi S, Babu KLG (2012) Effect of pomegranate and aloe vera extract on streptococcus mutans: An in vitro study. Dental Hypotheses 3:99