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## Experimental Study of Inhibitory Effect of the Grapefruit Seed Extract Mouthwash on the Streptococcus Mutans and Actinobacillus Actinomycetemcomitans in Vitro

Author: [LiuJianDang](#)

Tutor: [HuangXiaoJing](#)

School: [Fujian Medical](#)

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### Abstract

Dental caries and periodontal diseases are infectious diseases caused by plaque. [Streptococcus mutans](#) (*Streptococcus mutans*, S.mutans, referred to S.mutans) and [Actinobacillus actinomycetemcomitans](#) (*Actinobacillus actinomycetemcomitans*, Aa) is an important component of dental plaque bacteria present in the oral form of plaque biofilm. *Streptococcus mutans* is a major cariogenic bacteria closely related to the incidence of dental caries, Aa is closely related with localized aggressive periodontitis and chronic periodontitis also have a certain relationship. [Grapefruit extract](#) (grapefruit-seed extract, GSE) is a natural plant extract, contains a large number of polyphenols with antioxidant capacity, in recent years, studies have found that it has good anti-bacterial, fungal, viral and parasitic insects role, but also has good biocompatibility. GSE All mouthwash using edible grade materials, excluding ethanol, chlorine, iodine, tetracycline, metronidazole, and other ingredients, no stimulation to the oral mucosa, it will not cause the surface of the teeth, mucosal surface staining, but will not cause such as: The taste buds taste reduce, inhibit the secretion of saliva, dry mouth, burning, skin rashes and other adverse reactions. Task Force preliminary study found that the GSE mouthwash has a good plaque suppression, but its specific mechanism of action is not fully understood. In this study, by GSE mouthwash to *Streptococcus mutans* adhesion impact and its antibacterial effect on *Streptococcus mutans* and Aa and laser scanning confocal microscope (Confocal Laser Scanning Microscopy, CLSM) to observe the role S.mutans changes in the biofilm to investigate for the prevention and treatment of gingivitis, periodontitis and dental caries feasibility and mechanism to provide experimental basis for its application in the clinical treatment of oral diseases. The study included three-part test: experimental grapefruit extract solution *Streptococcus mutans* adhesion role purpose: research the GSE solution S.mutans smooth glass surface adhesion affect. Method: GSE Mouthwashes fold dilution method was diluted to different concentrations of GSE solution (1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1 / 256 Mouthwashes dope concentration), the variation of the amount of bacteria by measuring the optical density value of adhesion, reflecting the impact of the adhesion of *Streptococcus mutans* in a smooth glass surface; the morphological characteristics of the scanning electron microscope of *Streptococcus mutans* on the slide. Results: *Streptococcus mutans* adhesion on smooth glass with the the GSE solution concentration increased and decreased; [Scanning electron microscopy](#) showed that the matrix in the experimental group of bacteria, bacterial cells clear, blank group of bacteria parcel more amorphous material. Conclusion: a certain concentration of the GSE solution ( $\geq 1/128$  mouthwash dope concentration) inhibit *Streptococcus mutans* in the smooth glass surface adhesion. Experiment II Grapefruit extract solution *Streptococcus mutans* and *Actinobacillus actinomycetemcomitans* antibacterial action Objective: To study the GSE solution on the Aa and S.mutans the antibacterial activity, and to explore its antibacterial mechanism. Methods: the liquid dilution method the GSE solution of (1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024 rinses liquid oral fluid concentration) on the Aa and S.mutans the minimum inhibitory concentration and minimum bactericidal concentration; scanning electron microscope the GSE solution (1/16 mouthwash dope concentration) on the cell wall of *Streptococcus mutans*. Results: GSE solution Aa MIC 1/64 concentration of liquid mouthwash, MBC 1/32 concentration of liquid mouthwash; the S.mutans the MIC 1/32 mouthwash dope concentration, MBC 1/16 Mouthwashes dope concentration, scanning electron microscopy showed that the solution treated MBC concentration the GSE (equivalent Mouthwashes dope concentration of 1/16) *Streptococcus mutans* cell wall rupture, the contents of the overflow. CONCLUSION: GSE solution the Aa and S.mutans in vitro significant inhibitory and killing effect, the inhibition mechanism may be related to the GSE damage related to the integrity of the cell wall structure. Experimental laser confocal scanning microscopy grapefruit extract mouthwash anti-*Streptococcus mutans* biofilm effect purposes: To observe the effect of GSE rinse on the different stages of *Streptococcus mutans* biofilm and cetylpyridinium chloride mouthwash compared. Methods: In vitro formation 3H, 24h, 48h *Streptococcus* bacteria biofilm model GSE mouthwash, respectively, after 1min, dead bacteria and living bacteria in the biofilm of fluorescent dyes, CLSM observed, and mouthwash fluid before and after treatment of viable cells The percentage of change. For results: GSE mouthwash can reduce the viable percentage 3h *Streptococcus mutans* biofilm the outer and middle, as well as 24h, 48h biofilm percentage of viable cells of the outer layer; grapefruit and cetylpyridinium chloride in two mouthwash processing each period biofilm between the two layers of percentage of viable cells was no significant difference (P gt; 0.05) Conclusion: Grapefruit and cetylpyridinium chloride in two mouthwash effect of *Streptococcus mutans* biofilm role similar to the killing effect of the outer layer of the biofilm bacteria in each period, but only affect early (3h) S.mutans deep biofilm bacteria to survive.

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