



# Effects of Coconut Oil on *Streptococcus mutans* Biofilm Formation

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## BACKGROUND

Oil pulling is a traditional Indian folk remedy where oil is swished and forcibly pulled between the teeth to reduce plaque formation. It has the potential to prevent dental caries by disrupting *S. mutans* biofilm formation in part due to the presence of polyphenols found within coconut oil. Coconut oil specifically contains gallic acid, ferulic acid, quercetin, methyl catechin, dihydrokaempferol and myricetin glycoside.

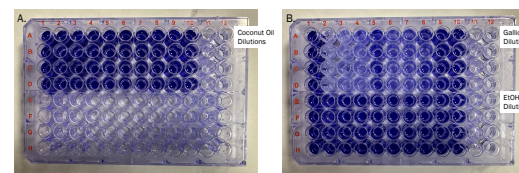
It was anticipated that coconut oil and gallic acid would have bactericidal effects on *S. mutans* as well as cause disruption of biofilm formation at the level of adhesion based on evidence provided by previous studies conducted on coconut oil and various polyphenols found within coconut oil.

The purpose of this study was to investigate the effects of coconut oil and gallic acid on *Streptococcus mutans* biofilm formation.

## METHODS

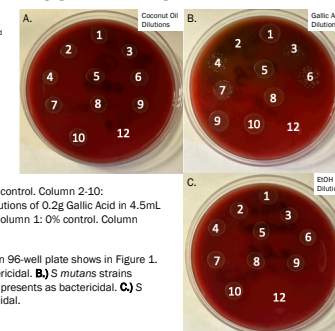
*S. mutans* strains were cultured using tryptic soy broth (TSB) in an atmosphere of 95% air/5% CO<sub>2</sub> at 37°C. The bacteria were then treated with dilutions of coconut oil and gallic acid ranging from 1:10-1:10,000 in TSB supplemented with 1% sucrose (TSBS) for 24 h in sterile 96-well flat-bottom microtiter plates. The solutions tested were 4 g coconut oil in 5 mL TSBS, 0.2 g gallic acid in 0.5 mL ethanol with 4.5 mL TSBS, and a control made of 0.5 mL ethanol with 4.5 mL TSBS. After incubation, the absorbance of each well in each plate was determined at 595 nm to establish the MIC, the planktonic unbound cells were then gently aspirated and transferred to a new 96-well plate and the absorbance at 595 nm was determined to assess the planktonic growth. The biofilm on the initial 96-well flat-bottom microtiter plate was then gently washed twice with saline, fixed with 10% formaldehyde (22), washed twice with saline, and stained with 0.5% crystal violet for 30 min. After washing the biofilm three times with saline, crystal violet was then extracted from the biofilm cells by incubation for 1 h with 200  $\mu$ l of 2-propanol. The extract was then diluted 1:5 with 2-propanol and the absorbance was read at 490 nm with 2-propanol used as the blank control.

### FIGURE 1: 96-WELL MICROTITER PLATE

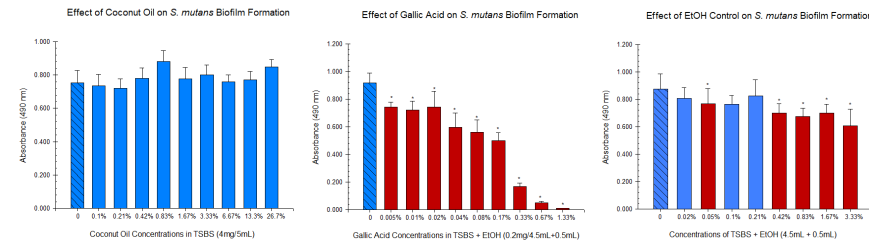


**Figure 1: A)** Rows A-D: *S. mutans* strains treated with dilutions of 4g Coconut Oil in 5mL TSBS. Column 1: 0% control. Column 2-10: 1:10-1:10,000 dilutions in TSBS. Column 12: Sterility control. **B)** Rows A-D: *S. mutans* strains treated with dilutions of 0.2g Gallic Acid in 4.5mL TSBS with 0.5mL EtOH. Rows E-H: *S. mutans* strains treated with dilutions of 4.5mL TSBS with 0.5mL EtOH. Column 1: 0% control. Column 2-10: 1:10-1:10,000 dilutions in TSBS. Column 12: Sterility control.

### FIGURE 2: MBC



**Figure 2:** Minimum bactericidal concentration (MBC) on blood agar plates. Numbers correspond to columns in 96-well plate shown in Figure 1. **A)** *S. mutans* strains treated with dilutions of 4g Coconut Oil in 5mL TSBS. No concentration present as bactericidal. **B)** *S. mutans* strains treated with dilutions of 0.2g Gallic Acid in 4.5mL TSBS with 0.5mL EtOH. Only 1.33% concentration in well 2 presents as bactericidal. **C)** *S. mutans* strains treated with dilutions of 4.5mL TSBS with 0.5mL EtOH. No concentrations present as bactericidal.



## RESULTS

Coconut Oil Biofilm: Concentrations of 0.83%, 26.7%, and 3.33% coconut oil had significantly higher biofilm than 0% control (p<0.05).

Coconut Oil Total Absorbance: All concentrations had significantly higher total absorbance than 0% control (p<0.005).

Gallic Acid Biofilm: All concentrations (0.005% to 1.33%) of gallic acid had significantly lower biofilm than 0% control (p<0.001).

Gallic Acid Total Absorbance: Concentrations 0.02% to 1.33% had significantly lower total absorbance than 0% control (p<0.004); concentrations 0.67% and 1.33% had significantly lower total absorbance than 0% control (p<0.002).

EtOH Biofilm: 0.05% and 0.42% to 3.33% ethanol had significantly lower biofilm than 0% control (p<0.05).

EtOH Total Absorbance: 0.42% and 3.3% had significantly lower total absorbance than 0% control (p<0.05).

## DISCUSSION

The results of this study do not support coconut oil inhibiting *S. mutans* biofilm formation. No MBIC or MBC were identified with the coconut oil concentrations used in this study. It is important to consider that there were difficulties identifying a detergent that could successfully dissolve coconut oil in TSBS. Various solvents were utilized including: DMSO, Tween 20, and ethanol, however none were successful. The coconut oil continued to separate from the TSBS after mixing, this could account for the lack of inhibition seen in biofilm formation. More studies need to be conducted after identifying a solvent capable of dissolving coconut oil in TSBS.

The results of this study do support the polyphenol gallic acid inhibiting *S. mutans* biofilm formation. The MBIC for gallic acid is greater than the 1.33% concentration used in this study. Since gallic acid is present in coconut oil, there is potential for coconut oil to inhibit *S. mutans* once a solvent that can dissolve it is identified. The ethanol control completed in this study shows that ethanol does inhibit *S. mutans*, however it inhibits at a much lower concentration than what was used to dissolve the gallic acid in TSBS at a 1.33% concentration.



## REFERENCES

