

# Evaluation of the Antibacterial Activity of Arabica Cold Brew and Arabica Spent Coffee Grounds on *Streptococcus mitis*

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Spent coffee grounds or coffee waste pose a growing environmental problem as it can harm the ecosystem by releasing greenhouse gasses into the atmosphere during their decomposition process. On the other hand, spent coffee ground extracts have been shown to contain polyphenols, trigonelline, phenolic acids, and melanoidins which show strong antibacterial activity toward Gram+ bacteria and yeast. Given its activity towards Gram+ bacteria, this study seeks to find an alternative use of spent coffee grounds, aiming to mitigate their environmental impact. This research focuses on the investigation on whether arabica spent coffee grounds could be used as a natural alternative to iodine mouthwash solution, a commonly used product to kill dental plaque bacteria. Arabica coffee extract was prepared by a 24-hour cold brew procedure then spent coffee grounds extract was produced from the Arabica coffee waste. To obtain results, the antibacterial activity of both spent coffee grounds and coffee on *S. mitis* was analyzed using the well diffusion test and inhibition zones were measured and compared. The caffeine contents of the Arabica coffee grounds were quantified using HPLC. From our quantitative analysis, the spent arabica coffee grounds collected from the cold brew procedure consist of less caffeine, compared to that of the cold brew, but still showed antibacterial activity towards *S. mitis*. The inhibition zone of the spent coffee grounds was significantly lower than cold brew coffee but still comparable to iodine mouthwash solution. Our novel findings indicate that spent coffee grounds can be used as a natural antibacterial and an alternative to povidone-iodine mouthwash which leads to reduction of environmental harm from coffee waste.

**Keywords:** Spent coffee grounds, *Streptococcus mitis*, cold brew, povidone iodine mouthwash, arabica coffee grounds, environmental waste

## Introduction

Coffee, one of the most widely consumed beverages globally, generates significant waste in the form of spent coffee grounds (SCG)<sup>1</sup>. Each year, the global coffee industry produces around 23 million tons of SCG as a byproduct of the brewing process<sup>2</sup>. When discarded, each ton of waste can emit up to 340 cubic meters of methane, a potent greenhouse gas, contributing to environmental pollution and landfill overflow<sup>3</sup>. As coffee consumption and waste continue to rise, finding ways to reutilize SCG is crucial for reducing their environmental impact.

Several studies in the past have shown that coffee has health benefits since it contains natural antibacterial properties that could fight against Gram+ bacteria<sup>4</sup>. Coffee or cold brewed coffee is said to have antibacterial properties since it contains trigonelline, caffeine and  $\alpha$ -dicarbonyl compounds<sup>4</sup>. A study in 2006 reported that coffee polyphenols, particularly chlorogenic acid, showed inhibitory effects against *Helicobacter pylori*, a pathogen associated with gastric ulcers and cancer<sup>5</sup>. Moreover, according to a recent study, Arabica spent coffee grounds as well as Robusta spent coffee grounds showed an inhibitory activity against *S. aureus* and *E. coli* at coffee waste concentrations of

1.0mg/mL<sup>6</sup>. These findings suggest that spent coffee grounds (SCG) or coffee has a strong potential to become a sustainable source of these bioactive compounds against both Gram+ and Gram- bacteria.

Although several studies have highlighted the antibacterial activity of coffee, only a few have investigated the potential of reusing SCG as antibacterial agents. In the site PubMed, when the keywords “antibacterial activity of coffee grounds” were searched, only 15 papers were shown in the past 10 years. However, when the keywords “antibacterial activity of spent coffee grounds” were searched, only 8 appeared in the last 10 years. Exploring this potential could offer a sustainable use for coffee waste, transforming an environmental burden into a beneficial resource.

Due to the increased global consumption of coffee daily and its active antimicrobial activity, coffee could potentially act as a natural alternative to povidone-iodine solution to kill harmful mouth bacteria. Povidone-iodine solution is commonly used in mouthwash to kill a wide range of bacteria, viruses, and simple organisms<sup>7</sup>. An example of a common mouth bacteria, *Streptococcus mitis*, is used in this paper in order to determine the antimicrobial activity of SCG and coffee grounds. *Streptococ-*

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*S. mitis* has a significant role in invasive infections especially those with poor oral care. When it travels to other organs from the oral cavity, it can cause invasive diseases such as endocarditis, enteritis, and meningitis<sup>8</sup>. While povidone-iodine solution could kill *S. mitis*, with overuse, it could have corrosive effects due to its iodine content<sup>9</sup>. Using cold brew or coffee grounds as alternatives could be safer for everyday use. Additionally, studying whether SCG could be a sustainable alternative to povidone-iodine solution would reduce harmful environmental impacts mentioned before.

According to the antibacterial activity of coffee grounds at 1.0mg/ml in past studies, this could hypothesize that both SCG brew and cold brew would have antibacterial properties against *S. mitis*<sup>6</sup>. Therefore, the objective of this study is to investigate the antibacterial activity of SCG from cold brew as an alternative to povidone-iodine solution against *S. mitis*. Povidone-iodine solution acts as the comparison to provide an understanding of their potential as natural antibacterial agents. Using the well diffusion method, different concentrations of cold brew, SCG and povidone-iodine solution were used in order to study the zone of inhibition of each agar plate. The results of this paper were based on the quantitative data obtained and concludes the potential of using SCG as an alternative to povidone-iodine solution.

## Materials and Methods

Aceh Gayo Arabica coffee beans were bought from Nagadi Coffee and ground coarsely using a home coffee grinder. Cold brew method was used in which the coffee was brewed in 2-8°C for 24 hours in water. Moreover, the cold brew method was taken from the common cold brew recipe from the New York Times and the standard ratio of coffee to water was 1:5. Since previous studies never used cold brew, a maximum concentration of 200 mg/ml was chosen. Initial stock solution of cold brew 200mg/ml was prepared by mixing 20g of ground coffee with 100 ml distilled water. This cold brew was then filtered using coffee filter paper to obtain the 200mg/ml cold brew stock solution and the spent coffee ground (SCG). Then a serial dilution of the cold brew was done to prepare these concentrations: 100mg/ml, 75mg/ml, 50mg/ml and 25 mg/ml, using distilled water. To make 200mg/ml SCG brew stock solution, 20g of SCG was cold brewed in 100 ml distilled water. Another set of serial dilutions of SCG brew, using distilled water, was prepared: 200 mg/ml, 100mg/ml, 75mg/ml, 50mg/ml and 25 mg/ml.

Commercial 1% povidone-iodine mouthwash (mouthwash) contains 10 mg/ml povidone iodine. From the mouthwash bottle, a serial dilution was done to prepare the following concentrations: 10 mg/ml, 5mg/ml, 2.5 mg/ml, 1 mg/ml and 0.5 mg/ml, using distilled water.

The *Streptococcus mitis* (NCIMB 13770) was supplied by PT. Embrio Biotekindo (MBrio) and cultured following MBrio's

culture certificate in Sheep Blood Agar (SBA). Aseptically, 0.1 ml of TSB media was added into the bacteria strain ampule. Sterile cotton swab was used to homogenize the mixture then the mixture was inoculated on the SBA petri dish. This petri dish was put into an anaerobic jar and incubated in 37°C for 24 hours. One loopful of 1 ml inoculum was mixed with 10 ml of 0.9% NaCl. The concentration of the solution was measured by the absorbance of the solution using a UV/Visible spectrophotometer at 580 nm. A UV/visible spectrophotometer measures the amount of light passing through the sample. If the absorbance is higher, this means that the sample is at a higher concentration. On inhibition testing by the USP <81> guidelines, at 580 nm, this sample proved its concentration with %T of 25%. After testing, 1ml of inoculum was added into 100 ml of SBA to make SBA+inoculum culture stock.

The susceptibility of *S. mitis* to cold brew, SCG brew and oral mouthwash were assessed by using the well diffusion test. The controls were the type of cork borer, type of agar dish, location of methodology, type of bacteria. For each testing of different solutions (mouthwash, SCG cold brew and cold brew), it was repeated with 3 replicates each. The povidone-iodine mouthwash solution was used as a comparator between the other 2 solutions. This purpose was to see whether the coffee solutions had a similar, more, or less antibacterial activity compared to an existing mouthwash based on the results of the well diffusion tests.

A stainless-steel cork borer with an inner diameter of 3.6 mm was used to make the wells. *S. mitis* agar dish was prepared by adding 4ml of SBA+inoculum onto a layer of solidified 21ml SBA. After bacteria and SBA solidify, 4 wells were made on each bacteria agar (one well mouthwash was added into each well. One bacteria agar had one type of concentration, so each agar had 4 wells of the same concentration of a sample solution. The above tests were performed in duplo for each sample. A negative control plate was prepared. All agar plates were incubated in 37°C for 24 hours. Quantitative analysis was used by determining the zone of inhibition of each sample. The zone of inhibition was measured using a millimeter specific ruler. If there is a larger diameter, this means that the sample is suspected to have a higher antimicrobial effect. Minimum inhibitory concentration (MIC) of each sample could not be determined through qualitative analysis directly from the zone of inhibition since it is usually measured quantitatively using another test according to a recent study in 2024<sup>10</sup>.

Caffeine content analysis was carried out at PT Saraswanti Indo Genentech using HPLC-PDA. Caffeine contents were measured for the following coffee samples: cold brew 200mg/ml and 25mg/ml, and SCG brew 200mg/ml and 25 mg/ml.

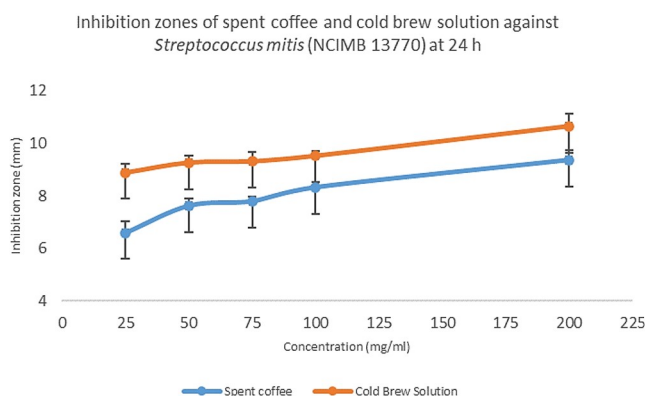
Statistical analysis was performed using paired t-test on Excel software on the well diffusion test result of coffee extract. The statistical analysis is an additional finding to the contents of the coffee extract to study how much caffeine is concentrated

in the SCG brew and cold brew solution. As previous studies have explained the antibacterial effects from caffeine, the analysis provided additional information to the reasoning behind antibacterial activity resulting from the well diffusion tests<sup>4</sup>.

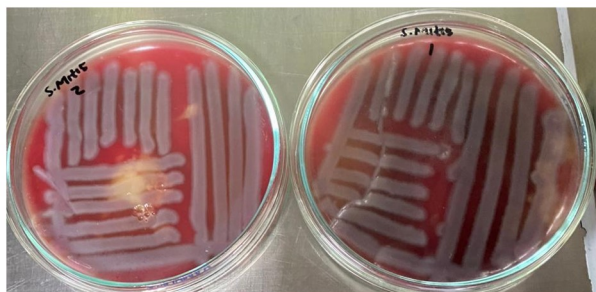
The trial was done in duplo so each sample had 8 wells. The inhibition zone of the SCG brew and cold brew solution were reported as the mean (with standard deviation). The significance level was set at  $p < 0.05$  and  $p < 0.05$  was considered to be statistically different to a significant degree.

## Results

As shown in table 1, the zone of inhibition of all the cold brew samples at all concentrations was larger than the SCG brew. However, SCG brew has a comparable zone of inhibition at high concentrations. The povidone-iodine solution was used as a comparator to both cold brew solutions. The evaluation using a paired t-test with  $\alpha = 0.05$  shows that the SCG brew solution significantly inhibits all concentrations compared to the cold brew solution ( $p < 0.05$ ). Inhibition is the lowest at concentration of 25 mg/ml for both cold brew and SCG brew.



**Fig. 1** Inhibition zone profile of cold brew solution and spent coffee ground brew solution against *Streptococcus mitis* at 24 h.



**Fig. 2** Profile of *Streptococcus mitis* growth for inoculation

*S. mitis* agar dishes were tested in 3 different solutions. The SCG cold brew solution and cold brew solution had 5 different concentrations at (a) 25 mg/mL, (b) 50 mg/mL, (c) 75 mg/mL, (d) 100 mg/mL, and (e) 200 mg/mL. The Povidone Iodine Mouthwash solution used as comparator was tested at smaller concentrations of (a) 0.5 mg/mL, (b) 1.0 mg/mL, (c) 2.5 mg/mL, (d) 5.0 mg/mL, and (e) 10.0 mg/mL, as shown in Fig. 3.

## Discussion

In this research, the antibacterial inhibitory effects of Arabica coffee cold brew, Arabica spent ground coffee brew and oral mouthwash at various concentrations on the growth of *S. mitis* were determined using the well diffusion method. Antibacterial inhibitory effects were indicated with the existence of a clear zone around the well. Due to limited resources of extraction equipment, home coffee grinder and coffee filter were used and the simplest method to gain the most caffeine extract was chosen, which is cold brewing<sup>11</sup>.

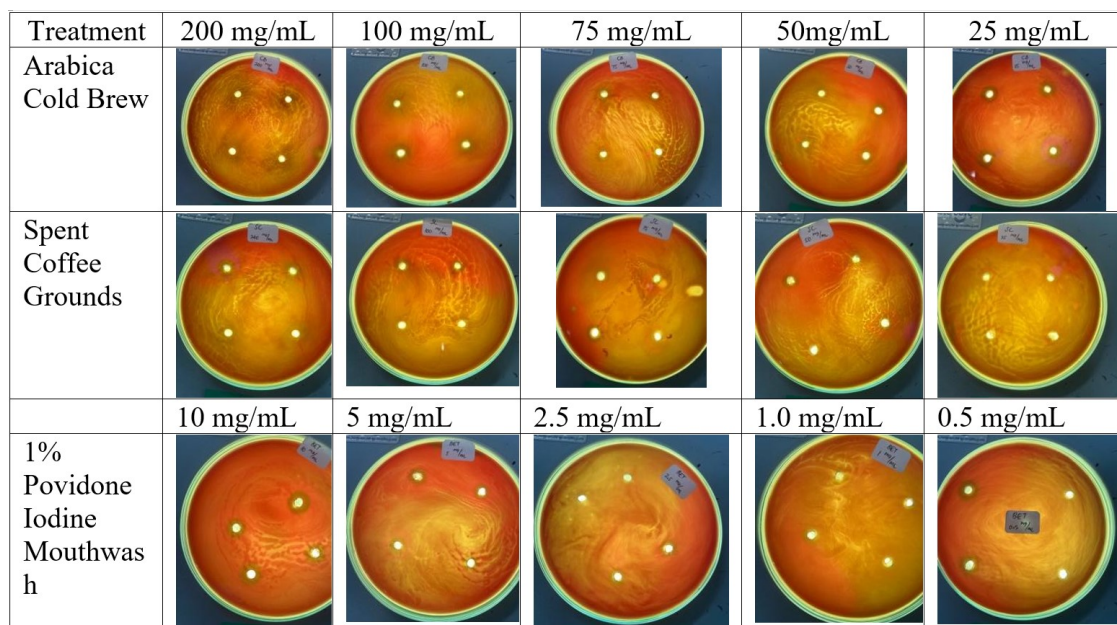
The results shown in Table 1 shows that all samples of Arabica coffee cold brew, Arabica spent ground coffee brew and oral mouthwash were able to inhibit the growth of *S. mitis* at different levels. As seen on Fig 1, we can see that there is a significant difference between the inhibition zones of cold brew than those of spent coffee grounds.

HPLC-PDA analysis (Table 2) revealed that Arabica cold brew at 200mg/mL has a higher concentration compared to SCG brew while at 25mg/ml concentrations, cold brew and SCG brew had almost the same caffeine content. This shows that caffeine is highly likely not the main component for antibacterial activity. According to Gaul & Donegan, the components of coffee that allows it to contain antibacterial activity are trigonelline, chlorhexidine and fluoride. However, since resources were limited, these other components in the coffee solutions could not be determined and only caffeine concentrations were measured.

A study comparing the antibacterial components of green coffee beans, roasted coffee and spent ground coffee showed that spent ground coffee has higher content of total phenolic compounds compared to roasted coffee grounds<sup>6</sup>. This may suggest that caffeine may not be the main component that acts as an antibacterial agent according to a study in 2004<sup>12</sup>. These two studies imply that although spent coffee grounds (SCG) have less caffeine than coffee grounds, it may still contain phenolic compounds that provide its antibacterial properties. Despite the limitations to these studies, spent coffee grounds still had activity against *Streptococcus mitis*, showing that they have the potential to be used as beneficial agents instead of harmful waste. According to Table 1, cold brew consistently showed a higher zone of inhibition compared to spent coffee grounds which strongly suggests that there is a certain component in

**Table 1** Inhibition zones of spent coffee ground brew solution, cold brew solution, and mouthwash solution against *Streptococcus mitis* (NCIMB 13770) at 24 h

Concentration (mg/ml)	Diameter Mean (mm) ± SD		Mouthwash Concentration (mg/ml)	Diameter Mean (mm) ± SD
	Spent Coffee	Cold Brew Solution		
25	6.59±0.44	8.88±0.33	0.5	8.86±0.54
50	7.62±0.26	9.26±0.26	1.0	7.61±0.25
75	7.79±0.18	9.31±0.36	2.5	7.84±0.18
100	8.32±0.19	9.52±0.17	5.0	7.84±0.15
200	9.36±0.39	10.65±0.47	10.0	9.94±0.65



**Fig. 3** The susceptibility of *Streptococcus mitis* to coffee extract and mouthwash solution.

normal cold brew that enhances the antibacterial activity which could be the trigonelline, chlorhexidine and fluoride<sup>12</sup>.

The antimicrobial activity of cold brew and SCG brew is shown to be potentially similar to povidone-iodine at certain concentrations. Looking at Table 1, cold brew has larger inhibition zones than SCG brew. At 100mg/ml, cold brew has a similar zone of inhibition compared to the povidone-iodine at 10mg/ml. Mouthwash (1% povidone iodine) is usually used by swishing 15ml of the solution in the mouth for 30 seconds without any dilution, so the normal concentration used is 10 mg/ml<sup>7</sup>. Moreover, according to the NYT, the typical cold brew drink has a ratio of 1:5 coffee to water<sup>13</sup>. Since the cold brew solution was made at this ratio, at the concentration of 100 mg/ml cold brew, it is comparable to the concentration typically used in mouthwash. This finding suggests the strong potential of cold brew exhibiting antibacterial activity at real-life usage levels

Although SCG brew has smaller inhibition zones than cold brew, SCG can still be comparable to povidone-iodine. At 200mg/ml, the SCG brew has a comparable zone of inhibition

with the povidone iodine solution at 10mg/ml. Even at a higher concentration, this level is still based on real-life usage of coffee. Overall, it proves that spent coffee grounds, at higher concentrations, have a potential to have similar antibacterial effects to povidone-iodine solution.

All diameters were measured after 24 hours of incubation while usually neither mouthwash or coffee are kept in the oral cavity for 24 hours. To better mimic real situations, further study needs to be done when the coffee solution is in immediate contact with the mouth bacteria. In order to compare the antibacterial effects with mouthwash, a mouthwash gargle is used for around 30-60 seconds. Thus, a further study needs to be conducted when the coffee solution is in contact with bacteria for 30-60 seconds.

## Conclusion

In summary, this study revealed a novel use of coffee and spent coffee ground, which is an antibacterial activity against Strepto-

**Table 2** Caffeine concentration of coffee extract

Concentration (mg/ml)	Caffeine Content (mg/ml)	
	Spent Coffee	Cold Brew Solution
200	2.142±0.0014	0.722±0.0007
25	0.106±0.0002	0.097±0.0005

coccus mitis with antimicrobial activity and that antimicrobial activity was comparable to that of marketed mouthwash and gargle 1%. The next step is to investigate the properties of spent coffee grounds that allow for antibacterial activity so the precise active biomolecule can be extracted properly. Moreover, a further action towards designing a commercial product is to study different preparations of spent coffee grounds to ensure longer stability and homogeneity of its contents, as well as make it more consumer friendly. Despite this, this paper concludes that the usage of spent coffee grounds as mouthwash could be a potential alternative to reduce coffee waste which leads to reduction of carbon emission.

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