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ANTI MICROBIAL ACTIVITY OF CAFFEINE EXTRACT AGAINST ORAL PATHOGENS (STREPTOCOCCUS MUTANS) - AN IN-VITRO STUDY

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ABSTRACT

BACKGROUND:

Caffeine is consumed daily by humans and people tend to miss out on its medicinal properties such as inhibition of bacterial growth and micro-organisms. There are less studies conducted to confirm its medicinal properties overall. In most of the countries the daily consumption of caffeine is high. Caffeine has proven to have some microbial activity as per other studies. Hence more studies can be conducted targeting specific oral pathogens and using caffeine to see the antimicrobial, antibacterial and other key properties. Studies have been conducted with caffeine to treat periodontal and gingival disease.

OBJECTIVE:

The aim of this study was to determine the antimicrobial activity of caffeine against streptococcus mutans.

MATERIALS AND METHODS:

Caffeine was extracted from roasted coffee beans and was sterilised by an autoclave and stored. 1ml from the sample autoclaved was taken in 3 cuvettes. 1 cuvette being the control (saline). McFarland standard was used to adjust the turbidity of bacterial suspension. 10 ml of *S.mutans* was added to each cuvette, from the cuvette it was added to the culture plate (Manitol Salt agar). After 5 minutes, the culture plate was incubated at optimum temperature. After 24 hours the culture plates inoculated with *S.mutans* and caffeine extract were examined and colonies formed were counted.

RESULTS:

Antimicrobial activity by caffeine was observed with the increase in concentration and the antimicrobial activity was minimal in lower concentrations.

CONCLUSION:

Caffeine extract has shown antimicrobial activity against *streptococcus mutans*. It was confirmed after reading the culture plates for CFU (Colony forming units).

Keywords: Caffeine, antimicrobial activity, *streptococcus mutans*

INTRODUCTION:

Coffee is a worldwide food product with a total production of 8,700,000 kg in 2013 (International Coffee Organization ICO, 2013). Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid present in nearly 100 plant species **[1]**. Its main natural sources are tea (*Camellia sinensis* L.), coffee (*Coffea arabica* L.), cocoa (*Theobroma cacao*), and maté (*Ilex paraguariensis*). Caffeine's effects on human health have been broadly studied **[2]**. Besides that, its antimicrobial properties have been examined against: human pathogens like *Staphylococcus aureus, Klebsiella pneumonia,* and *Pseudomonas aeruginosa*, constituents of natural human microflora such as *Escherichia coli, Streptococcus oralis,* and *Propionibacterium acnes*, but also *Pseudomonas fluorescens* and *Bacillus subtilis* that are present in terrestrial and aquatic habitats. Research findings also show caffeine as a potential tool for the control of diseases caused by plant-pathogenic bacteria, especially under storage conditions **[3]**.

Coffee is one of the most widely consumed beverages in the world. Hence, understanding its composition and actions on the human body are of scientific benefit. Coffee bean extract has been known to have antimicrobial effects against both Gram-positive and Gram-negative bacteria as far back as 1989 **[4]**. Coffee has demonstrated significant

antibacterial properties against the cariogenic bacteria Streptococcus mutans and Streptococcus mitis and has also been found to be effective against the periodontal pathogens *P. gingivalis* and *P. intermedia*, as well as *Candida albicans* **[5]**.

Coffee extract has demonstrated significant antimicrobial properties against various Gram-positive and Gram-negative bacteria. 0.2% chlorhexidine, a potent allopathic reagent, in the mouthwash form is considered the gold standard of chemical plaque control. A study was conducted by Yi TL *et al* (2016) to evaluate the antimicrobial efficacy of different concentrations of coffee extract with 0.2% chlorhexidine mouthwash on the following Gram-negative periodontal pathogens: *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans* under in vitro conditions. The study findings showed that Coffee at a concentration of 20% and 15% showed activity against *P. gingivalis, P. intermedia* and *A. actinomycetemcomitans. F. nucleatum* was resistant to all concentrations of coffee extract. The authors concluded that coffee extract possesses antimicrobial activity against the various periodontal pathogens though not as efficacious as the standard chlorhexidine. Commercially available coffee extract does indeed possess antimicrobial activity against the periodontal pathogens *P. gingivalis, P. intermedia* and *A. actinomycetemcomitans* **[6-26]**.

AIM OF THE STUDY:

The aim of the study was to explore the antimicrobial activity of caffeine against oral pathogen (Streptococcus mutans)

MATERIALS AND METHODS:

Caffeine was extracted from roasted coffee beans from Al Hasa, Saudi Arabia, these extracted caffeine powder was mixed with 100 ml distilled water and stored **(Figure 1)**. This was then autoclaved and sterilised thoroughly. 1 ml from sample autoclaved was taken in 3 cuvettes separately **(Figure 2)**. 1 Cuvette being the control (saline). McFarland standard was used to adjust the turbidity of bacterial suspension. 10 ml of *S.Mutants* cultivated at Microbiology lab Saveetha dental college was added to each cuvette **(Figure 3)**. From the cuvette it was added to the culture plate (Manitol salt agar). After 5 minutes, the culture plates were incubated at an optimum temperature with the help of an incubator for 24 hours. After 24 hours the culture plates were inoculated with *S.mutans* and caffeine extract and were examined and colonies formed were counted with the help of colony counting app **(Figure 4)**.

Figure 1

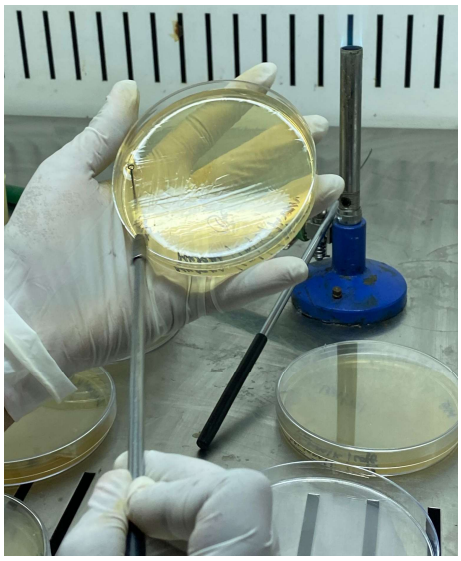

Figure 2

Figure 3

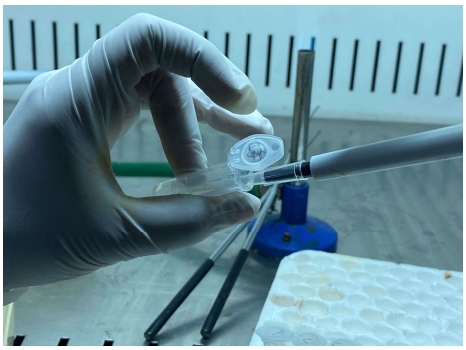

Figure 4

RESULTS:

The culture plates were analysed and explored visually and with the help of colony counter app, the CFU (Colony forming units) were counted. After counting the colonies formed by *S.mutans*, the culture plate with the highest concentration of caffeine had less *et al* colonies formed, whereas the culture plate with the lowest concentration of caffeine had comparatively more *S.mutans* colonies formed. Whereas the culture plate (test) with saline had the maximum number of *S.mutans* colonies formed with the number of 627. Seen in the below images **(Figure 5, 6)**. Culture plate number 3 had the minimum number of *S.mutans* colonies formed with the number of 258 having the highest amount of caffeine concentration. Hence, antimicrobial activity by caffeine was observed with the increase in concentration and the antimicrobial activity is minimal in lower concentrations.


Promega
Colony Counter Results
Mar 4, 2021 8:56 AM
Count:
627
Plate Set:
Unnamed Plate Set
Promega
Colony Counter Results
Mar 4, 2021 9:01 AM
Count:
312
Plate Set:
Unnamed Plate Set
Promega
Colony Counter Results
Mar 4, 2021 9:02 AM
Count:
305
Plate Set:
Unnamed Plate Set
Promega
Colony Counter Results
Mar 4, 2021 9:03 AM
Count:
258
Plate Set:
Unnamed Plate Set

Figure 5

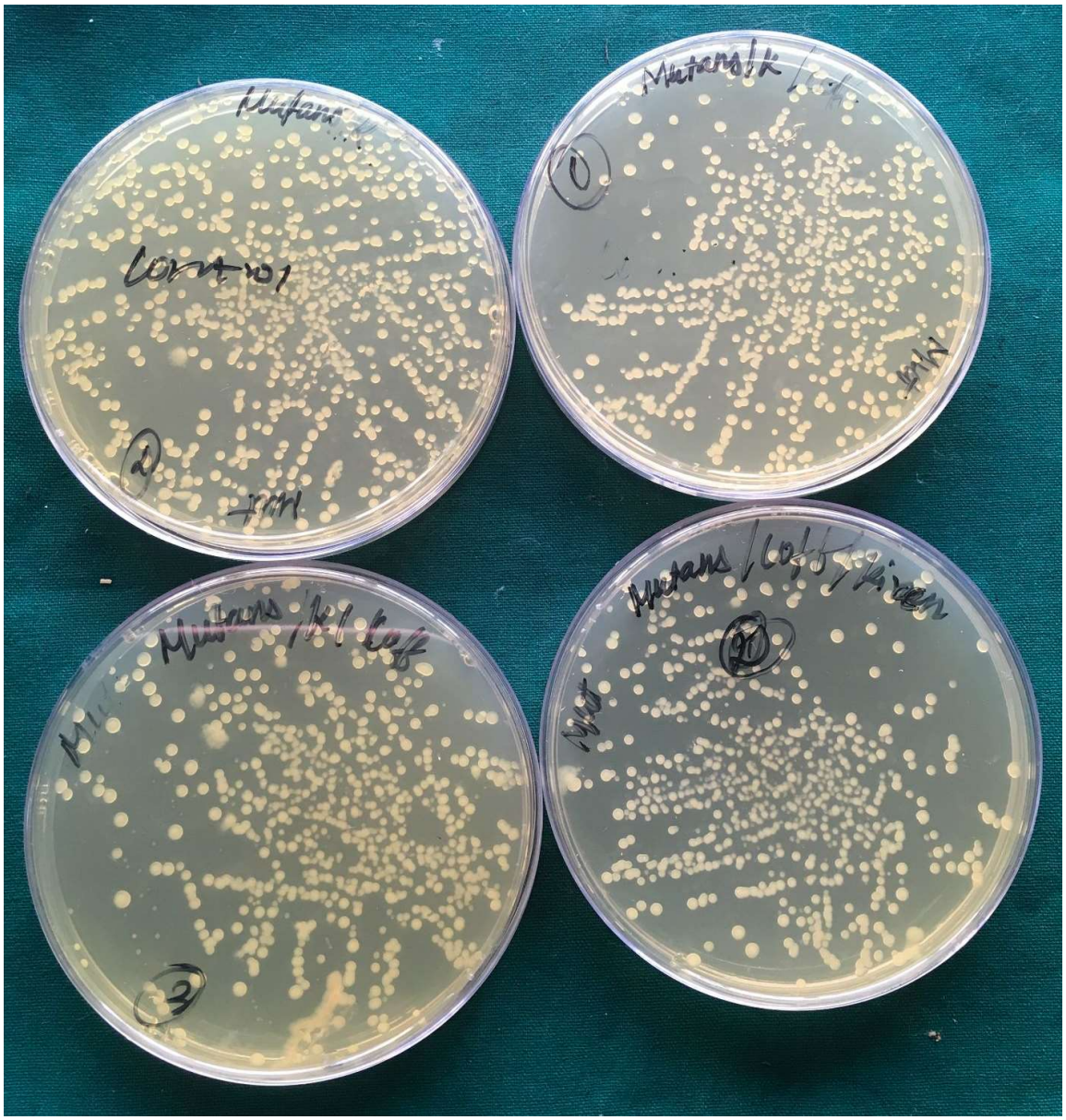

Figure 6

DISCUSSION:

This study was conducted to find the influence of natural coffee compounds, coffee extracts and increased levels of caffeine on the inhibition of *Streptococcus mutans*. The inhibition of *Streptococcus mutans* by *Coffea arabica* extracts incorporated or not with natural coffee compounds was investigated by the disk diffusion method. In our study, findings also showed caffeine at concentrations found in Arabica beverages inhibited *Streptococcus mutans* temporarily, whereas higher caffeine concentrations provided a stronger and longer lasting inhibition. The results obtained make coffee extracts potential inhibitors of

dental caries. It is anticipated that in the future, by determining the concentration of effective compounds, the antimicrobial activity of a certain type of coffee will possibly be predicted **[27]**.

In a study done by Almeida *et al*. on 'Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria' the in vitro antimicrobial activity of commercial coffee extracts and chemical compounds was investigated on nine strains of enterobacteria. The researchers concluded that concentrations of caffeine found in coffee extracts are enough to warrant 50% of the antimicrobial effect against *S. enterica*, which is relevant to human safety **[28]**.

Various plant and fruit extracts have been monitored recently to assess their potential against the common oral pathogens. An In-vitro Study on 'Antimicrobial efficacy of Punica granatum mesocarp, Nelumbo nucifera Leaf, *Psidium guajava* Leaf and *Coffea Canephora* Extract on Common Oral Pathogens' was conducted by Mehta VV *et al* **[29]**. The results showed that all the four extracts were found to be effective against *S.mutans* and *S.mitis*, with maximum efficacy against *S.mutans* and *S.mitis* displayed by pomegranate and lotus. Antifungal efficacy was demonstrated by coffee and pomegranate. Guava, lotus and coffee were effective against *P.intermedia*, while only coffee was found to be effective against *P. gingivalis*. The authors concluded that pomegranate, guava, lotus and coffee displayed significant anticariogenic effects while coffee was found to be most effective against periodontal pathogens as well as Candida albicans **[29]**.

Perhaps the most convincing evidence to date on the clinical effectiveness of coffee on periodontal disease comes from a longitudinal study by Ng *et al*. in which they observed a small, but statistically significant reduction in the number of teeth with periodontal bone loss. Moreover, it has also been concluded that though the benefits of coffee towards the periodontium may be currently unclear, another important finding is that its consumption is in no way harmful towards periodontal health **[30, 31]**.

The growth inhibition of common food-borne pathogens and food spoilage microorganisms by coffee extracts is also studied. Spent coffee showed antimicrobial activity, mainly against Gram-positive bacteria (*Staphylococcus aureus, Listeria monocytogenes*) and yeast (*Candida albicans*). Coffee extracts with concentrations less than 160 mg had anti-bacterial activity against Gram-negative strains **[32]**.

The need for this study is to find the antimicrobial activity of caffeine against *Streptococcus mutans*, so that it could be more used for clinical treatment purposes in dentistry. In the future further, more studies with a clinical trial and greater sample size are needed to determine the antimicrobial activity of caffeine against oral pathogens.

CONCLUSION:

Caffeine concentration in Arabica coffee could inhibit *Streptococcus mutans* temporarily and a stronger and long lasting effect could be achieved with higher caffeine concentrations. Coffee extracts could be potential inhibitors of dental caries. Coffee extract possesses antimicrobial activity against the various periodontal pathogens though not as efficacious as the standard chlorhexidine. From the lab study conducted, we can conclude that caffeine shows efficacy against *S.mutans* for its antimicrobial effects. Further studies to be conducted against oral pathogens for targeted treatment.

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