# Antimicrobial activity of Coffee on organisms causing Dental caries

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## Abstract

Dental biofilm constitutes an ecosystem of bacteria that produces acid from carbohydrate metabolism. From a biochemical point of view, the bacteria in the biofilm are always metabolically active, causing fluctuations in pH. This results in loss of minerals, leading to dissolution of the dental hard tissues and the formation of a caries lesion. Therefore, an important strategy for the prevention of dental caries is to reverse or halt the mineral loss. Researchers worldwide have been searching for alternatives to prevent the occurrence of dental caries. There has been an increased interest in the properties of some plant stimulant beverages, particularly chocolate, coffee and tea, which have demonstrated anti-cariogenic *in-vitro* and *in-vivo*activity.Coffee contains caffeineand trigonelline which contribute to flavor and aroma, may be responsible for antibacterial activity. The aim of this study was to evaluate the *in-vitro* antimicrobial activity of coffee-based solutions obtained by sublimation method on *Staphylococcus aureus,Bacillus and Candida albicans*.

**KEYWORDS:** Coffee, dental caries, antimicrobial activity, oral bacteria, caffeine.

## Introduction:

The oral cavity hosts a complex ecosystem composed of hundreds of different microbial species. Pioneer oral bacteria colonize biotic and abiotic structures, over time and following numerous specific interactions with other species, a climax community is reached. This bacterial proliferation is usually a consequence of a diet rich in sugars such as sucrose and/or of poor oral hygiene and may lead to the commonest oral pathologies, such as caries, gingivitisand periodontitis. Many strategies for reducing the accumulation of plaque have been proposed, ranging from the use of sugar substitutes to the use of vaccination against specific odonto-pathogenic bacteria (*e.g. Streptococcus mutans*) and antimicrobial agents such as antibiotics and antiplaque agents in mouth rinses and toothpastes.[9]

Dental caries yet remain a widespread public disease that highlight an urgent need to find new effective strategies. Researchers worldwide have been searching for alternatives to prevent the occurrence of this process. Mutans *Streptococci* are the main etiologic agent of caries, especially on smooth surfaces.[1] The adherence of bacterial cells to tooth surface is of great importance in the development of carious lesions and the interference of some of these mechanisms can prevent the formation of carious lesions. [4].In the last few years, there has been an increased interest in the properties of some plant stimulant beverages, particularly chocolate, coffee and tea, which have demonstrated anti-cariogenic in vitro and in vivo activity.[6]

Several studies have demonstrated the activity of natural plants on the dental biofilm and caries development. Few studies on antimicrobial and anti-cariogenic properties of coffee-based solutions are found in literature. Some components in coffee such as caffeine,non-

volatile organic acids, phenols and aromatic compounds are reported to have antimicrobial activity.

Coffee is the dried seed of the fruit originated from a tree of theCoffea genus, Rubiaceaefamily[1].Coffee contains several compounds which are known to affect human body chemistry. Now researchers are finding that coffee seems to have a range of health benefits, including reducing the risk of type 2 diabetes, gallstones, liver cancer, Parkinson's disease and Alzheimer's disease. It also increases the effectiveness of painkillers and is an antioxidant[6]

A six ounce cup of coffee contains 100-150 mg of caffeine.This compound was effective against Gram positive and Gram negative reference strains. The study data suggest that trigonelline,caffeine and other water-soluble compounds in coffee that contributes to the aroma and flavor of the beverage may be responsible for anti-adhesive and antimicrobial activity of coffee[12]. Nevertheless, researchers conclude “we can hypothesize that due to antibacterial and anti-adhesive activity, coffee might reduce the colonization of tooth surface of organisms causing dental caries and thus prevent tooth decay”.

The aim of this study was to evaluate the in vitro antimicrobial activity of coffee based solutions obtained by sublimation method on cultures of *Staphylococcus aureus, Bacillus and Candida albicans* .

**Materials and Methods**

The study involved the following steps

I. Growth and standardization of test cultures
II. Extraction and purification of the bioactive compound
III. Testing of inhibitory concentrations

I-Growth and standardization of test cultures

A.Preparation of Mueller Hinton broth;Beef infusion: 300gm;Acid hydrolysate of casein:17.5 gm;Starch :1.5 gm;Distilled water-1000ml,pH7.2

B.Testcultures:Following standard strains were used for screening of antibacterial and antifungal activities:

i. *Bacillus*-Gram-positive bacteria
ii. *Staphylococcusaureus*-Gram positive bacteria
iii. *Candidaalbicans*-Fungus

C. Inoculum preparation:Inoculum was standardized at$1x10^6$ CFU/ml

II Extraction of the bioactive compound and purification by sublimation

Extraction of aqueous coffee solution was carried out by Liquid-Liquid extraction and purification by sublimation**.** It is a technique used to selectively remove one component from a solution containing several dissolved components. The solution was extracted with another solvent capable of dissolving only the desired component which was more soluble in the extraction solvent. The two solvents chosen were immiscible when performing a liquid-liquid extraction. The extraction solvent helped in removing the desired component and the washing solvent helped in selectively removing the impurities.

Caffeine is an alkaloid,one of many naturally occurring nitrogenous bases. In a cup of coffee,caffeine is mixed with a variety of other components including acidic tannins that contribute to the dark colour of coffee.

i. To extract the caffeine without the carry-over of other components, sodium carbonate wasadded to ensure the solubility of tannins and other acidic components in the water layer.
ii. The pH of coffee was raisedto ensure the caffeine is in a free base form,one that is very soluble in organic solvents, thus caffeine gets extracted in iso-propanol.

The technique of sublimation is useful for obtaining compounds of high purity if they are amenable to this approach. Many organic compounds can be made to sublime under vacuum.

i. The vapor phase was condensed to a solid on a cold finger.
ii. Care was taken to ensure that the sample does not decompose when heating, also to minimize mechanical losses of the sample
iii. Sample purification was a necessary part of compound synthesis or extraction but purification techniques often result in significant product losses-it is difficult to recover all of the original mass of the compound in pure form after a purification procedure.

III-Determinationof Minimum Inhibitory concentration byTube method and study of anti-microbial sensitivity by Agar cup method.

Minimum Inhibitory Concentration (MIC)by Tube method:

i. Pure culture of *Bacillus, Candidaalbicans, Staphylococcusaureus* was grown in Mueller-Hinton broth.
ii. The culture was standardized using standard microbiological techniques to have a concentration of approx$10^6$ cells per milliliter.
iii. Coffee extract was diluted in sterile Mueller-Hinton broth to prepare different concentrations ranging from 2mg/ml to 10 mg/ml.
iv. After the antimicrobialagenti.e Coffee was diluted, 0.1 ml volume of the standardized inoculum was added to each dilution tube.
v. The inoculated, serially diluted coffeewas then incubated at an appropriate temperature for the test organism for a pre-set period,24 hours.
vi. After incubation, the series of dilution tubes were observed for microbial growth, indicated by turbidity and/or a pellet of microorganisms in the bottom of the tube.
vii. The last tube in the dilution series that does not demonstrate growth corresponds with the minimum inhibitory concentration (MIC) of the antimicrobial agent.

Antimicrobial sensitivity by Agar cup method.

i. The plates were inoculated by bulk seeding 0.5ml of culture in sterile 20 ml molten Mueller and Hinton medium.
ii. Cups were punched in plate using a cork borer. Different concentrations of coffee extract were added in the wells.
iii. The plates were placed in an incubator at 37°C for 24 hours.
iv. After 24hrs incubation, the diameter of zone of inhibition was measured and recorded in mm. The results are presented in the table.

**Results**

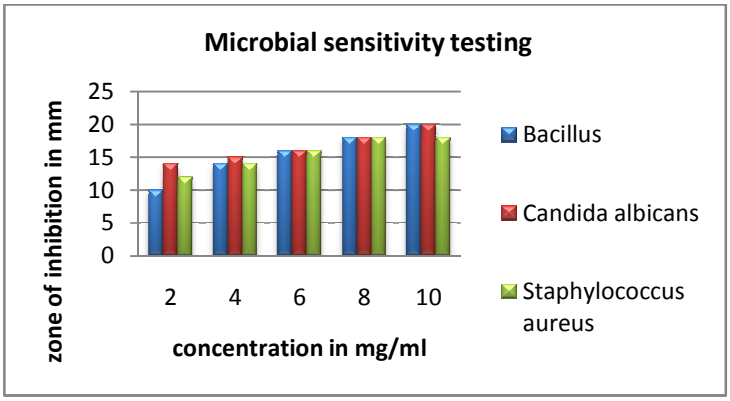
I-Minimum inhibitory concentration

| **Concentration (mg/ml)** | **Turbidity** | | |
|---|---|---|---|
| | *Bacillus* | *Candida albicans* | *Staphylococcus aureus* |
| **2** | + | + | + |
| **4** | + | + | + |
| **6** | + | + | + |
| **8** | + | - | - |
| **10** | - | - | - |

Key :+Growth - No Growth

II-Microbial sensitivity test

| **Concentration mg/ml** | **Zone of inhibition(mm)** | | |
|---|---|---|---|
| | *Bacillus* | *Candida albicans* | *Staphylococcus aureus* |
| 2 | 10 | 14 | 12 |
| 4 | 14 | 15 | 14 |
| 6 | 16 | 16 | 16 |
| 8 | 18 | 18 | 18 |
| 10 | 20 | 20 | 18 |




**Discussion**

The use of plant extracts with medicinal properties represents a concrete alternative for the treatment of different diseases. This includes the use of natural products as antimicrobial agents even though in the absence of scientific basis such practices may generate serious side effects. Regarding the use of coffee extracts, it could not be disapproved, since this beverage is part of habitual diet for many people, except for those who are sensitive to caffeine. There is no doubt that coffee is the most consumed beverage in the world after mineral water.In addition to its pleasant flavor, it has been considered as a potential functional food for its bio-pharmacological properties demonstrated in clinical and epidemiological researches.In the last decade, aseries of studies have been performed exploring the anti-

infective properties of coffee on different microorganisms,which can generate a significant improvement in managing several kinds of health disorders.

The main compounds responsible for such activity in roasted coffee extracts are chlorogenic acids, caffeic acid and caffeine.Other minor compounds are trigonelline,α-dicarbonyl compounds and protocatechuic acid.

According to our results,the minimum concentration of coffee extract that inhibited the growth of *Bacillus*was 10mg/ml,*Staphylococcusaureus* was 8mg/ml and *Candidaalbicans* was 8mg/ml.Antimicrobial sensitivity test also reflects the antimicrobial property of coffee extract on *Bacillus,Staphylococcusaureus* and *Candidaalbicans* showing as proportionate increase in inhibition zones with increasing concentration of coffee.

In view of the present data,a light roasted coffee aqueous extract can be considered as potential anticariogenic substance due to its capacity of preventing the growth of *Bacillus, Staphylococcusaureus* and *Candidaalbicans* and of inhibiting dental demineralization. However,as much as we have tried to a mimic a real situation, these results were obtained in an in vitro environment.Further in vivo studies should be elaborated with the aim of investigating new approaches for caries management such as the benefits of retention of this anticariogenic agent into dental biofilms.

**Conclusion**

Series of studies have been performed exploring the anti-microbial properties of coffee on different microorganisms *Bacillus,Staphylococcusaureus* and *Candida albicans*.Coffee extract demonstrated a significant inhibitory effect in both broth assay as well as Agar cup assay. In view of the presented data a light roasted Coffee aqueous extract can be considered as a potential antibacterial agent. Further *in- vivo/in vitro* studies should be elaborated with the aim of investigating a new approach for caries management such as the benefits of the retention of this antibacterial agent into dental biofilms. This proves an important strategy for the prevention of dental caries is to reverse or halt the mineral loss.

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