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The antibacterial activity of ethanolic extract of some Plant containing polyphenols component against *Streptococcus mutans* isolated from teeth caries

Sura Ihsan Abed Jabuk

University of Babylon, College of Science , Department of Biology, Hilla, Iraq

Abstract : Caries is still considered one of the main problems of public health and many researchers in the world have been searching for alternatives to prevent the occurrence of this process. *Streptococcus mutans* has been isolated and identified from dental caries. The colonies of bacteria were identified by simple biochemical tests. The inhibitory effect of alcoholic plant extracts (Coffee, Cacao, Green and Black tea) on *S. mutans* isolated were studied by using agar well diffusion method and turbidity analysis. Coffee and Green tea showed the maximum inhibition zone against *S. mutans* compare with Cacao and Black tea . the result showed the MBC of Coffee, Cacao, Green and Black tea against *S. mutans* was 200, 500, 300 and 400 mg/ml respectively and MIC was 600, 900, 700, 900 mg/ml respectively.

Key Word : Minimum inhibitory concentration , Minimum bactericidal concentration, Coffee, Cacao, Green tea , Black tea.

Introduction

Oral cavity of human supports life for many different species of microorganisms, but bacterial infection happened when these microorganisms permeate into the tissues or in case of lower host immunity. The display of bacterial infection of dental descent is permanently changing and is a measurable reflexing of new evolution of oral flora (1).

The *Streptococcus* bacteria, is the most common cause of tooth decay that are gram-positive, immotile, non spore formation , catalase negative and facultative anaerobic (2).

There is an urgent need for the prevention of tooth decay by prevention programs of oral diseases and promote personal health (3).

In addition to the daily cleaning of the teeth, there are certain nutrients from our diet helps to prevent tooth decay, including coffee, tea (black and green) and cocoa (4).

Coffee is contain extremely complex mixture of several hundred chemical materials that are either existed naturally or introduced in the coffee as a result of the roasting process. In addition to the impact of the alarm clock s as a result of contain caffeine they contain strong chemicals that are antioxidants such as acid alkloroujen, chlorogenic acid, who is in abundance in coffee and melanoidins. Fresh coffee contains strong antioxidants materials equal to the amount present in the three oranges (5).

Roasted coffee has anti-bacterial activity against gram positive and negative bacteria including *Str. mutans* , which is the main cause of tooth decay in humans. (6).

Both black and green tea contains catechins and flavinoids but in different studies found that black tea has more protective effects in the prevent the formation of biofilm on the tooth surface as compared to green tea. (7)

The effectiveness of tea to prevent biofilm formation belong to the active ingredients include: caffeine 4%, amounts of altheoberman, theophylline, alzanfein, fluoride and other factors such as polyphenolic compounds and tannic acid as well as it contains colorful materials, volatile oils and oxidative enzymes (8).

The seeds of the cacao contains plant oils called cocoa butter, also contains the alkaloid caffeine , alkaloid theobromine and low percentage of volatile oils in addition contain polyphenols and anti-oxidant material (4).

Researchers carried out test to measure levels of antioxidants in tea, cocoa, the result of the tests showed that cocoa contains twice amount of antioxidants material compared with tea (5).

In addition of cocoa there are many products of it such as chocolate, but the researchers say that drinking cup of cacao is the best way to take fully advantage of its health benefits, because the chocolate is rich in fat, chocolate which contains about 8 - 40 grams of fat, compared to 0. 3 grams in a cup of cocoa. (9).

This research aims to study the antibacterial activity of cocoa, black tea, green tea and coffee on *str. mutans* bacteria isolated from tooth decay.

Material & Methods

A- Isolation Method

The *Str. mutans* bacteria was isolated from teeth caries of the staff and students of Biology Department in College of Science, Babylon University by using sterile swab and were taken the samples to the microbiology laboratory 30 min after collection. Each sample was inoculated onto Muller Hinton agar with 5% sheep blood. plates were incubated aerobically at 37C for 24 hours, with further 24 hours incubation if there was no growth (10).

B- Steps of identification and maintenance of test organisms

Direct smears were prepared and stained with gram stain to seen morphological arrangement by using light microscope. The colonies of bacteria were identified by simple biochemical tests. All the pure cultures were stored and maintained in nutrient broth at 4°C for further use. (11).

C- Preparation of ethanolic extract

The Cocoa, Black tea, Green tea and Coffee were powdered mechanically and then immersed in 90% ethanol with volume (ml) three times of powder weight (g). After 24 h, solid fraction was removed while liquid fraction was transferred into rotary evaporator chamber. Evaporation was conducted at temperature of 60°C for 3.5 h. Ethanolic concentrate was subsequently vaccum dried at 40-60°C to produce solid particles, considered as Cocoa , Black tea ,Green tea and Coffee Ethanolic Extract. Analyte was prepared by diluting 2 g of Cocoa , Black tea ,Green tea and Coffee Ethanolic Extract in 2 ml hot sterile distilled water. After vigorous shaking, obtained solution was gradually diluted with sterile distilled water to obtain concentration of 1000, 500, 250, 125mg/ml (12).

D-Detection of Susceptibility to Antibacterial Agents:

Concentrations (1000, 500, 250 and 125 mg / ml) were prepared from each Plants extracts . Plants extracts concentration was loaded on sterile filter paper disc (5mm diameter) and applied on the surface of Muller -Hinton agar plates inoculated with bacterial isolates and incubated at 37°C for 24 h (13).

E-Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration(MBC)

To determine the MIC, a series dilution (1000, 900, 800, 700, 600, 500,400,300,200,100 mg/ml) were prepared from each plant extract. Each dilution added to the tube contain BHI medium broth which contains 1.5×10^8 CFU/ml bacterium. Simultaneously a tube containing 9 ml medium plus 1 ml of extract are for control extract and a tube containing 9 ml medium as well as 1 ml of bacteria's suspension were prepared as control bacteria. All test tubes were placed at 37°C for 24 hours. After the incubation period the tubes' inoculated turbidity were studied due to bacterial growth. (14). The extract dilution containing lowest concentration and maximum inhibition turbidity due to bacterial growth was considered as the MIC of that extract.

To determined MBC 1 ml was taken from each tube were mixed with 20 ml of BHI agar in Petri dishes and incubation for 24 hours. Dilution plates containing the lowest concentration of the extract and no colonies of bacteria was found were conceder as MBC of that extract (14)

F-Statistical analysis:

Statistical analysis was performed using SPSS statistical computer software

Result& Discussions :

The result showed the Coffee and Green tea more effect than Cacao and Black tea against *Str. mutans* ($p<0.05$) Table (1) . Similar result was obtain with Studies done by Subramaniam *et al* and Balappanavar *et al* (15,16). while Naderi *et al* present the antibacterial activity of black tea more effect on *S. mutans* than green tea (13) . Drinking green tea shows promise when it comes to preventing dental caries as it fights bacteria and lowers the acidity of saliva and dental plaque (14).

Scientific studies and research recently revealed that green tea useful in the treatment of many diseases, because it contains large amounts of antioxidants and many important minerals and vitamins, especially vitamin e (17).

Also present the green tea able to stop the erosion of dental process, when tooth erosion process caused by a type of bacteria that feed on sugar to produce acids such as lactic acid and these acids dissolve tooth enamel. In addition, green tea contains natural substance «fluorine» that strengthen teeth and prevent erosion. Thus tea not only prevents adhesion of *streptococci* to tooth surface, it also prevents its coaggregation (18).

The authors observed that coffee compounds may inhibit the formation of glucosyltransferase by cariogenic bacteria. They further speculated that this inhibition probably was due to the action of polyphenols (19).

The biological properties of polyphenols include antioxidant, anticancer, and anti-inflammatory effects. The antimicrobial effects of polyphenols have also been widely reported as their ability to inactivate bacterial toxins(20) .

Table(1) The diameter of inhibition zone (mm) of the plant extract against *streptococcus mutans*

Concentration mg /ml Plant extract	1000	500	250	125
Coffee	40	32	29	20
Black Tea	12	9	5	2
Green Tea	35	28	23	15
Cacao	15	18	9	5

$p<0.05$ significant difference

Present the MBC of Cacao and Black tea was 900 mg/ml , 600 mg/ml of Coffee and 700 of Green tea against *Str.mutans* Table (2) . Another study present MIC and MBC for Coffee were 7 mg/mL and 160 mg/mL, against *Str. mutans* (21) . While Smullen *et al* present MIC 4mg/ml and MBC 64mg/ml against *Str.mutans* (22).

Studies have shown that chocolate helps protect teeth from decay, which is very effective in prevent formation biofilm layer and that cocoa beans that are the main component of chocolate, inhibit the growth of bacteria that cause tooth decay.(23)

They discovered that cocoa shell bead, which is the outer part of it that are not utilized in the manufacture of chocolate, has anti-bacterial properties in the mouth and can fight against biofilm layer and destructive factors with great effectiveness (24).

Table (2) The Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration(MBC) mg/ml of the plant extract against *streptococcus mutans*

Plant extract	MIC	MBC
Coffee	200	>600
Cacao	500	>900
Black tea	400	>900
Green tea	300	>700

The researchers noted the cocoa, coffee , green and black tea are very effective in prevent tooth caries so that some scientists suggested to add some of their components to mouthwashes and toothpaste (23).

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