Effect of Black Cardamom Extracts on Mutans Streptococci in Comparison to Chlorhexidine Gluconate and De-ionized Water (*In Vitro* Study)

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ABSTRACT

Background: Spices and herbs have been used by many cultures to enhance the flavor and aroma of food and for their medicinal value. Black cardamom is one of these spices widely used in cooking because of its unique taste and powerful flavor. The aim of study was to test the effect of black cardamom on *Mutans Streptococci* in comparison to chlorhexidine gluconate (0.2%) and de-ionized water.

Materials and methods: Dried fruits of black cardamom were extracted by using alcohol (70% ethanol). Saliva was collected from seven volunteers. Agar well technique with different concentrations of black cardamom extracts was used to test the sensitivities of *Mutans Streptococci,* as well black cardamom extracts effect on viable counts of *Mutans Streptococci.*

Results: *Mutans Streptococci* was sensitive to different concentrations of alcohol extracts of black cardamom in vitro starting with (5%) to (40%) using agar well diffusion technique. Black cardamom was effective in inhibition of *Mutans Streptococci* but still weaker than chlorhexidine gluconate 0.2%. Highly significant reduction in the counts of bacteria was reported with cardamom extracts and CHX in comparison to neutral control after 2 hrs.

Conclusions: Black cardamom showed an effect on *Mutans Streptococci* but still less than CHX.

Key words: *Mutans Streptococci*, black cardamom, chlorhexidine, de-ionized water. **(J Bagh Coll Dentistry 2016; 28(4):153-157)**

INTRODUCTION

Dental problems are very common among population and consider as the fourth most frequent illness condition, behind headache, high blood pressure and colds [(1)]. The most common oral diseases affect oral cavity is dental caries and periodontal disease [(2)].

The oral cavity is a complex system which can be altered by diverse factors including poor oral hygiene and diet, stress and systemic diseases which enhance the colonization by pathogenic bacteria and the formation of biofilm and their metabolism of fermentable carbohydrate leads to the formation of acids, biofilm imply the involvement of microbiological species most commonly *Mutans Streptococci* [(3)].

Mutans Streptococci is anon-motile, gram positive bacteria and considered as primary causative agent of initial caries [(4)]. The *Mutans Streptococci* are from the family: *Lactobacillaceae*, genus: *Streptococci*. *S. mutans*, occupies a substantial proportion of the microbiota that integrates the cariogenic biofilm, and their participation in the etiology of dental caries is very important [(5)].

Mechanical removal of dental plaque biofilm is a main factor in the prevention of oral diseases and might be associated with using agents which act particularly against cariogenic bacteria [(6,7)].

The optimal intervention for oral disease is not universally affordable because of high costs and limited resources, the use of chemical agents associated with many side effect [(8,9)].

Herbal medicines are increasingly used as dietary supplements for treatment against different human disorders with their safety and efficacy [(10,11)]. The antibacterial effect of herbs and spices in medicine can be justified according to their easy absorbability by the body without having any adverse effects if taken in appropriate amount [(12,13)].

Black cardamom also known as Amommum subulatum (A. subulatum) is a small herb which has strong aromatic smellswith a camphor-like flavor in the family Zingiberaceae [(14)], thus nice flavor and aroma can stimulate the taste buds when used in savory dal preparations and rice [(15)]. Away from being used in a wide variety of sweets and beverages, it is also a common ingredient of Pan Masala and Garam Masala [(16)].

Black cardamom has many benefit in several dental disorders related to oral health such as teeth and gum infection [(17,18)]. Antimicrobial effect of black cardamom in vitro had been documented as effect against *Streptococcus mutans*, *Staphylococcus aureus*, *Lactobacillus acidophilus* and *Candida albicans* [(19)].

The aim of this study was to test the effect of black cardamom extract on sensitivity and growth of *Mutans Streptococci* in comparison to chlorhexidine gluconate (0.2%) and deionized water.

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MATERIALS AND METHODS

Stimulated salivary samples were collected from seven healthy person from Baghdad–University, College of Medicine by chewing a piece of arabic gum, after disappearance of salivary foam, 0.1 ml of saliva is transferred to 0.9 ml of sterile phosphate buffer saline (PBS) of pH 7.0-7.2 for microbiological analysis.

Ten-fold dilution were performed, the inoculum was withdrawn from (10^{-3}), 0.1 ml was taken and spread in duplicate on the mitis salivarius bacitracin (MSB) agar which is the selective media for *Mutans Streptococci*. The plates were incubated anaerobically for 48 hrs., then aerobically for 24 hrs. at 37°C. The colonies of *Mutans Streptococci* were determined according morphological characteristic and Gram's stain [20].

Biochemical test for bacterial identification was done using cysteine tripticase agar media, thus test the ability of bacteria to ferment sugar. Agar well technique was applied to study the antibacterial effects of different concentrations of alcoholic black cardamom extracts (5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%), compared with chlorhexidine 0.2% as a control positive and de-ionized water as control negative on MHA media. These experiments were conducted on 7 isolates of *Mutans Streptococci*. MHA used in this experiment which were prepared and sterilized previously in sterile petri dish plates. To several plates *Mutans Streptococci* inoculum was spread, left at room temperature for 20 minutes.

Several wells of equal size and depth were prepared in each agar plate; each well was filled with 0.1 ml of the test agent. Plates were left at room temperature for 1 hour then incubated aerobically for 24 hrs. at 37°C. Zone of inhibitions which is clear zone of no growth of the bacteria were measured across the diameter of each well by using a ruler.

The viability counts of *Mutans Streptococci* from broth media, to which different concentrations of alcoholic extracts of black cardamom, CHX 0.2% and de-ionized water were added have been estimated in comparison to the control (broth and bacteria only). The procedure was carried on 4 isolates of *Mutans Streptococci*, the concentrations were 10%, 15%, 20%, 25%, 30%, 35%, 40% of alcoholic cardamom extracts. Brain heart infusion broth (BHI) was used which distributed in test tubes by 8.9 ml to each tube, 0.1 ml of the test agents was added to each tube except the control which was broth and bacteria only [21].

From the control tube 0.1ml was transferred to 0.9 ml of sterile normal saline. Ten-fold dilution was performed, from dilution 10^{-5}, 0.1 ml was taken and spread in on MSB agar plates, incubated anaerobically at 37°C for 48 hrs. the colony-forming unit per milliliter (CFU/ml) was counted. This value was considered as the initial count of bacteria. Study and control broth cultures were incubated aerobically for two hours at 37°C.

From each broth 0.1 ml was transferred to 0.9 ml of sterile PBS (pH 7.0) and ten-fold dilutions were performed. From dilution and 10^{-5}, 0.1 ml was taken and spread on MSB agar plates and was incubated anaerobically for 48 hrs. at 37°C. The colony-forming unit per milliliter (CFU/ml) was counted.

Data processing and analysis were carried out by using SPSS program version 19, which provide mean and standard deviation of the variables in the study and analysis of variance (ANOVA) for testing the significant differences among means of different groups. LSD was used with ANOVA with significant result, paired t-test also used to compare the difference between two means of the same group. The analysis was accepted at $P < 0.05$, as the limit of significance, when $P < 0.01$ were regarded as highly significance.

RESULTS

Black cardamom extracts were prepared by a method for alcoholic extract, with a dark brownish black, oily and viscous consistency (Figure-1).

Mutans Streptococci colonies are spherical or ovoid in shape with raised or elevated surface, light blue in color, and about 1-2 mm in diameter (Figure-2).

Mutans Streptococci cells were gram positive, spherical or ovoid in shape, arranged in short or medium length non-spore forming chains as shown in (Figure-3).

Mutans Streptococci colonies have the ability to ferment mannitol. A positive reaction was indicated by changing in color from red to yellow by formation of acid after incubation (Figure-4).

In the sensitivities of *Mutans Streptococci* to different concentrations of black cardamom, CHX and deionized water in vitro, the diameter of inhibition zone was found to increase as the concentrations of extracts increased. De-ionized water showed no zone of inhibition while CHX showed the highest zones of inhibition compared to black cardamom extracts, as one way ANOVA was performed among black cardamom, CHX and D.W. (Table-1).

The counts of *Mutans Streptococci* were tested used black cardamom, CHX, de-ionized water and control (broth+*MS*) (Table-2). Paired t- test was used to compare between initial count of bacteria

and count of bacteria after 2 hrs. Statistically, highly significant increase in number of bacteria was recorded after 2 hrs (Table-3).

Figure 1: Black cardamom extract product

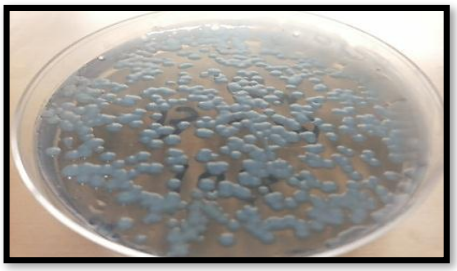

Figure 2: *Mutans Streptococci* on MSBA.

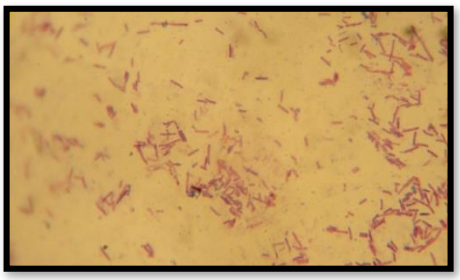

Figure 3 Gram's stain for *Mutans Streptococci* cell

ANOVA test and LSD among agents in comparison with initial counts and counts after 2 hrs. were also showed (Table-4) and (Table-5).

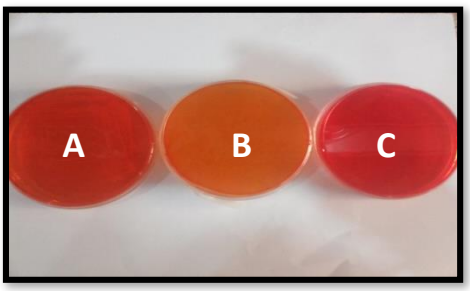

Figure 4: Biochemical identification for *Mutans Streptococci*
A: Positive control group (agar and bacteria without mannitol).
B: Study group (agar and mannitol inoculated with MS).
C: Negative control group (agar and mannitol without bacteria).

Table 1: *Mutans Streptococci* inhibition zone to different concentrations by different agents using (Agar well diffusion methods).

Agents	No.	* Mean	± S.D	ANOVA test
CHX	7	19.14	1.06	
Large cardamom 5%	7	0.57	0.78	
Large cardamom 10%	7	2.42	0.78	
Large cardamom 15%	7	4.85	1.21	F=165.16
Large cardamom 20%	7	6.62	1.16	P= 0.00
Large cardamom 25%	7	7.85	1.67	df= 9
Large cardamom 30%	7	8.08	1.08	HS
Large cardamom 35%	7	8.14	1.67	
Large cardamom 40%	7	9.00	0.81	
D.W.	7	0	0	

***(mm)**

Table 2: Effect of different concentration of large cardamom extracts, CHX and de-ionized water on viable count of *MS* X10^5 in vitro.

Agents	No.	*Mean	±SD	ANOVA test
Alcoholic extract 10%	4	130.00	4.71	F= 8.775 P=0.02 df= 8 S
Alcoholic extract 15%	4	100.00	0.41	
Alcoholic extract 20%	4	85.00	5.81	
Alcoholic extract 25%	4	73.87	7.50	
Alcoholic extract 30%	4	45.00	12.24	
Alcoholic extract 35%	4	41.25	10.30	
Alcoholic extract 40%	4	28.00	5.71	
CHX	4	7.00	3.46	
D.W.	4	212.50	14.43	

***(CFU/ml)**

Table 3: Initial count of *MS* and count of it after 2 hrs. (X10^5).

	*Mean	±SD	Paired t-test
Initial count	135.0	28.86	**t= -14.20 P=0.00 df=3 HS**
Count after 2 hrs.	237.5	14.43	

*** (CFU/ml)**

Table 4: ANOVA test among agents with initial counts and counts after 2 hrs.

	Agents	ANOVA test
Initial count	**Black cardamom CHX D.W.**	F= 27.71 P= 0.00 HS
Count after 2 hrs.		F= 143.55 P= 0.00 HS

Table 5: LSD between agents in comparison with initial counts and counts after 2 hrs.

Large Cardamom Conc.	Initial count			Count after 2 hrs.		
	*Mean Difference	P-value	Description	*Mean Difference	p-value	Description
10%	-5.00	0.64	NS	-107.50	0.00	**HS**
15%	-35.00	0.00	**HS**	-137.50	0.00	**HS**
20%	-50.00	0.00	**HS**	-152.50	0.00	**HS**
25%	-61.25	0.00	**HS**	-163.75	0.00	**HS**
30%	-90.00	0.00	**HS**	-192.50	0.00	**HS**
35%	-93.75	0.00	**HS**	-196.25	0.00	**HS**
40%	-107.00	0.00	**HS**	-209.50	0.00	**HS**
CHX	-128.00	0.00	**HS**	-230.50	0.00	**HS**
D.W.	77.50	0.00	**HS**	-25.00	0.03	**S**

***(CFU/ml)**

DISCUSSION

The primary role of the salivary *Mutans Streptococci* is to predict the future incidence of dental caries [22]. *Mutans Streptococci* play an important role in the development and progression of dental caries [23,24].

Stimulated saliva samples were superior for the reason that they yielded higher levels of *Mutans Streptococci* with lower sample variance than with unstimulated saliva [25]. In the biochemical identification, yellow color indicated that enough acid was produced by fermentation of the sugar (manitol) to lower the pH to 6.8 or less. Black cardamom with alcoholic extract with different concentrations was shown an effect on *Mutans Streptococci* by tested with two experiments (sensitivity and viable count).

The effect of black cardamom increased as the concentration increased. The results showed that black cardamom extract was able to inhibit the growth of *Mutans Streptococci* by affecting on zone of inhibition which increased as the

concentration increased from 5% to 40, at the same time the viable count showed a highly significant reduction of *Mutans Streptococci* with concentrations 10% to 40% compared to the control.

This finding was in coincidence with other studies [19,26]. All concentrations of black cardamom extract were shown lower inhibition zone than CHX, it's a potent antibacterial agent practically against *Mutans Streptococci* [27].

The de-ionized water had zero effect on the bacteria appearing by absence of inhibition zone. The results of black cardamom in the present study and its low price compared with green cardamom and more popularity in many countries in the world; give a great hope to import and use black cardamom in the life as food additive as well as in many medical and dental applications, not as a substitution for green cardamom but as a unique spice used in many spicy dish. Also there extractions were used as an ingredient in many antibacterial materials like mouth wash and dentifrices.

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