Antibacterial effect of cardamom and black tea aqueous extract on mutans streptococci in comparison to chlorhexidine (in vitro study)

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ABSTRACT

Background: Antimicrobial agents have been considered as having potential for the prevention of dental caries. This study aimed to test the effect of different concentrations of cardamom and black tea extracts on the sensitivity and growth of salivary mutans streptococci in comparison to chlorhexidine gluconate (0.2%) in vitro.

Materials and methods: In this study. Mutans streptococci were isolated from saliva of 34 healthy people (aged between 22-40yrs). The bacteria was isolated, purifiedand diagnosed according to morphologicalcharacteristic and biochemical tests. Aqueous extracts of cardamom and black tea were prepared. Different concentrations of extracts were prepared and estimated in gm/ 100ml deionized water. The agar diffusion technique was used to determine the antibacterial activity of cardamom and tea extracts in which the inhibition of bacteria growth by different concentrations of extracts was measured by diameter of inhibition zone in millimeter. The viable count was measured in different concentrations for both types of extracts on comparison to chlorhexidine 0.2%.

Results: The result showed that the mutans streptococci is more sensitive to tea extract than cardamom one, where the mean value of diameter of inhibition zone was higher by tea extract than cardamom type in all concentrations and chlorohexidine0.2% is more effective than both extracts. For viable count no statistical significant difference between two extract types at concentration of 40% but there are a high statistical significant difference for other concentrations, where the chlorhexidine is moreeffective than tea type and the last one is more effective than cardamom type with p value 0.05 .

Conclusions: Cardamom and black tea have antibacterial effect against Mutans streptococci; the accused factor of dental caries.

Key words: Cardamom, black tea, Mutans streptococci, antibacterial. (J Bagh Coll Dentistry 2013; 25(3):158-164).

INTRODUCTION

Finding healing powers in plants is an ancient idea. People on all continents have long applied poultices infusions of hundreds, if not thousands of indigenous plants, dating back to prehistory [1].

Cardamom is the third most expensive spice in the world (after saffron and vanilla), and the high price reflects the high reputation of this most pleasantly scented spice [2]. It refers to several plants of the genera Elettaria and Amomum in the ginger family Zingibreaceae. Both genera are native to India; they are recognized by their small seed pod, triangular in cross-section and spindle shaped, with a thin papery outer shell and small black seeds. Elettaria pods are light green while Amomum pods are larger and dark brown commonly known as black cardamom [3, 4]. The content essential oil in the seeds is strongly on storage conditions, but may be as high as 8%. In oil were found α-terpineol 45%, myrcene 27%, limonene 8%, menthone 6%, β-phenllandrene 3%, 1,8-cineol 2% and heptane 2% [5].Cardamom has a strong, unique taste, with an intensely aromatic, resinous fragrance. Both forms of cardamom are used as flavorings in food and drink, as cooking spices and as a medicine. It is a common ingredient in Indian cooking, and is often used in backing in Nordic countries such as in the Finnish sweet bread pulla or in Scandinavian bread Julekake. 60% of the world production is exported to Arab countries, where the large part used to prepare coffee and tea. Individual seeds are sometimes chewed, in much the same way as chewing gum to neutralize the toughest breath odors [6].

In traditional medicine it is used to treat infections in teeth and gums, to prevent and treat throat troubles, congestion of the lungs and pulmonary tuberculosis, inflammation of eyelids and totreat digestive disorders such as stomach-aches, constipation, and dysentery. It also used to break up kidney stones and gall stones, and was reportedly used as an antidote for both snake and scorpion venom [7].

Cardamom is best stored in pod form because once the seeds are exposed or ground they quickly lose their flavor. The seeds show a loss of about 40% of the essential oil per year [8].

One of the most popular, widely used plants all over the world is the tea plant (Camellia sinesis; Fam; Teacea) [9]. The tea plant is a shrub or semi tree belonging to the transtroemia with alternative evergreen leaves. The fresh leaves pickets from the tea plant are processed into varicose kind of tea according to the means of manufacturing methods but the three main types of tea can be classified as black, green and oolong tea.There are more than 500 various chemical elements contained in tea. Black tea has many

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more components than green tea partly because of the oxidation process that occur during fermentation [10]. Tea consists of caffeine 1%_4%, tannin 10_24%; fluoride, cadimium, cobalt, vitamins and volatile oil have been identified among the elements in tea [11].

Tea plant is considered to be one of the most important medicinal plants due to its various beneficial effects. Tea has antioxidant effect, antimutagenic activity; antimicrobial effects, anticarcinogenic property, reduce blood pressure and the possibility of coronary heart disease. Tea improves the cognitive and psychomotor performance [12].

The antibacterial activity (especially anticariogenic activity of cardamomand tea) is going to take place in this study aiming tobenefit from tea and cardamom extract to control and prevent dental caries in the future. Dental caries is a microbial disease affecting mineralized tissues of teeth [13]. Mutans streptococcus (MS) is considered to be the most accused factors to the initiation and progression of dental caries [14].

MATERIALS AND METHODS

Preparation of aqueous extracts

Cardamom was purchased from the market. Ground into fine powder in an electrical mixer. 100g of the finely powdered cardamom mixed with one liter of de- ionized water and kept in a water bath at 60 C for five hours, then filtered through filter paper. The extract was then left to dry at 40º C in hot air oven for evaporation of water. The extract was preserved in a refrigerator until use [15].

The commercial type of tea was used in this project supplied from local supermarket. The aqueous extract was prepared by 100g of dried leaves of black tea were infused in 500ml. of boiling distilled water, and left to cool at room temperature. Agitation of the infusion with magnetic stirrer had been done alternatively. Then the infusion was filtered by filter paper (wattman No. 1) and the residue discarded. The extract left to dry in a petridish at room temperature, the resulted powder kept in tightly closed glass container in refrigerator until used for different concentrations[1].

Isolation of mutans streptococcus

Stimulated saliva was collected from 34 healthy individual (aged 22-40yrs). Saliva was homogenized by vortex mixer for 2 minutes and serial dilutions were prepared by using normal saline then the appropriate dilutions from saliva for each microbial type was inoculated on Mitis-Salivaris Bacitracin Agar (MSB agar), selective media for MS; by taking 100ml from dilution (10^{-2},10^{-4}) and spread on plates of MSB agar by sterile microbiological spreader after thatincubated anaerobically by using gas pack supplied in anaerobic jar for 48 hours at 37 C followed by aerobic incubation 24 hours at 37 C[16]. The colonies on MSB agar were studied under microscope and gram stain was used followed by biochemical test to identify the isolates by catalase test and carbohydrate fermentation test[17].Different concentrations of cardamom and black tea extracts were prepared, 40%, 80%, 120% and 160%. The agar diffusion technique was applied to study the antimicrobial effect of these concentrations on thegrowth of MS and colony counts.

Statistical analyses

Data were analyzed using SPSS program version 20.Data were present in simple measures of mean and standard deviation, the significance of difference between studied variables was tested using student t- test for comparing between two means of independent groups. ANOVA used to test the difference among groups and Dunitti test used to compare the results of two groups with control one. P value less than0.05 was used as the level of significance.

RESULTS

The result revealed a high statistical significant difference by independent t- test between the two types of extracts. (p value $\leq$ 0.05). The result showed that the micro-organism is more sensitive to tea extract than cardamom one, where the mean value of diameter of inhibition zone was higher by tea extract than cardamom type in all concentrations as seen in Table 1and figure 1.

The result of ANOVA test illustrates a high statistical significant differences (p value 0.05) regarding the mean diameter of inhibition induced by three types of extracts at all concentrations ,where the result showed that the chlorohexidine (CHX) is more effective than tea extract and the latter one is more effective than cardamom one, as seen in Table 2. When a comparison of inhibition zone induced by two types of extracts (tea and cardamom) and control group (CHX) by Dunetti test revealed high statistical significant differences in all tested concentrations (p value 0.05) where the chlorhexidine is more effective than tea and cardamom extracts in all concentrations as seen in Table 3. The figures 2 and 3 showed a positive linear correlation between the diameter of inhibition zone and different concentration of each extract, where the mean of diameter of inhibition zone increased by increasing the concentration.

Counts of M.S were tested in the presence of different concentrations of cardamom and tea aqueous extract. The result revealed no statistical significant difference between two extract types at concentration of 40% but there are a high statistical significant difference for other concentrations with p value less than 0.05 as seen in Table 4. The result demonstrated a high statistical significant difference among three groups at different concentrations regarding the mean viable count of MS, where the result revealed that the chlorhexidine is more effective than other groups with p value 0.05 .When the Dunneti test to compare the result of viable count by two extract types with the control group (chlorhexidine),the result revealed a high statistical difference between each type of extract and control group in different concentrations with p value 0.05.Table 5 and 6.

DISCUSSION

Scientific analysis of plant components follows a logical pathway. Initial screening of plants for possible antimicrobial activities typically being by using crude aqueous or alcoholic extraction and sometimes can be followed by various organic extraction methods. The aqueous extract of plants is considered to be the best way for the extract activity, in addition , the amateur herbalist will be able to prepare this extract easily[(1)].

The sensitivity of mutans streptococci to different concentrations of cardamom and black tea aqueous extract in comparison to 0.2% chlorhexidine gluconate antimicrobial agent was tested. The zone of inhibition were found to increase when the concentration of cardamom and tea extract increased; but for tea extract zone of inhibition were higher than that seen for cardamom one. This may indicate that the sensitivity of Mutans streptococci to higher concentrations of aqueous extract of tea was more than that of cardamom extract. It could due to that the antimicrobial constituents of tea was in much more amount than that obtained from cardamom extract.

In the present study effects of aqueous extract of cardamom and tea on the viable count of mutans streptococci were tested. Both types had antibacterial activity against mutans streptococci because they were able to reduce the viable cell count profoundly. This is a desirable property for anticariogenic agent. The results showed that no statistical significant difference between two extract types at concentration of 40% but there are a high statistical significant difference for other concentrations. CHX had the greatest effect in comparison to the both.

Mouth washes are used to clean and deodorize the oral cavity. Generally, they contain antibacterial agents and are most often used for their deodorizing, refreshing and antiseptic effects [(9)]. The results of this study indicate that cardamom and tea had highly significant antimicrobial activity. Such antimicrobial property is a desirable property for mouth washes. These findings were in coincidence with other studies [(9,18-21)].

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Table1. Mean diameter of inhibition zone/mm by two types of extracts.

Conc.	Type of extract	No.	Mean diameter of inhibition zone/mm	±S.D.	t- test	p-value
40%	Tea	34	6.6	.7851	7.186	HS
	Card	34	5.3	.6974		
80%	Tea	34	7.1	.7634	8.022	HS
	Card	34	5.7	.6599		
120%	Tea	34	7.6	.7855	9.041	HS
	Card	34	6.1	.6213		
160%	Tea	34	8.1	.6937	11.157	HS
	Card	34	6.4	.5841		

HS=highly significant

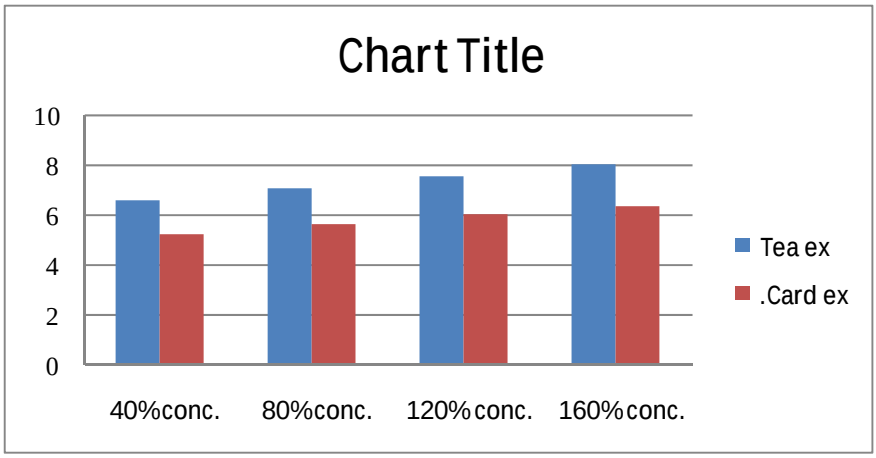

Figure 1: 1Mean diameter of inhibition zone of MS/mm by two types of extracts in different concentrations.

Table 2. Mean diameter of inhibition zone by different types of extracts at different concentrations

Concentration /extract type		No.	Mean Of inhibition zone/mm	±S.D.	95% Confidence Interval for Mean		F test	p-value
					Lower Bound	Upper Bound		
40%	Tea	34	6.6	.7851	6.403	6.950	318.061	HS
	Card	34	5.3	.6974	5.139	5.626		
	CHX	34	10.6	1.1500	10.258	11.060		
80%	Tea	34	7.1	.7634	6.904	7.437	275.080	HS
	Card	34	5.7	.6599	5.552	6.013		
	CHX	34	10.6	1.1500	10.258	11.060		
120%	Tea	34	7.6	.7855	7.385	7.933	234.952	HS
	Card	34	6.1	.6213	5.889	6.323		
	CHX	34	10.6	1.1500	10.258	11.060		
160%	Tea	34	8.1	.6937	7.899	8.383	217.456	HS
	Card	34	6.4	.5841	6.202	6.610		
	CHX	34	10.6	1.1500	10.258	11.060		

HS=highly significant

Table 3. Compares of mean of inhibition zone between two types of extracts and control group (CHX)

Dependent Variable	(I) Type of extract	(J) Type of extract (Control group)	Mean Difference (I-J)	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
40%	Tea	CHX	-3.9824*	.001	-4.472	-3.493
	Card	CHX	-5.2765*	.001	-5.766	-4.787
80%	Tea	CHX	-3.4882*	.001	-3.969	-3.007
	Card	CHX	-4.8765*	.001	-5.357	-4.396
120%	Tea	CHX	-3.0000*	.001	-3.479	-2.521
	Card	CHX	-4.5529*	.001	-5.032	-4.074
160%	Tea	CHX	-2.5176*	.001	-2.978	-2.057
	Card	CHX	-4.2529*	.001	-4.713	-3.793

CHX=chlorhexidine

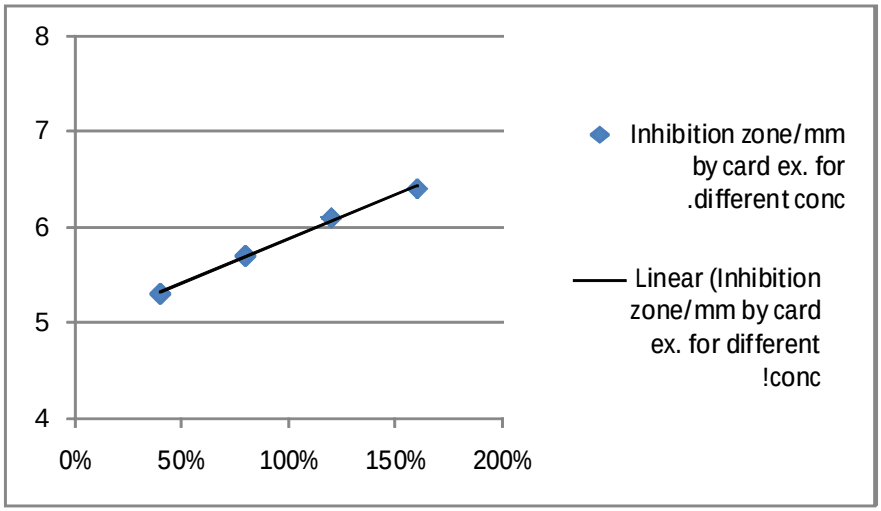

Figure 2. Correlation between different concentrations of cardamom extract and mean diameter of inhibition zone

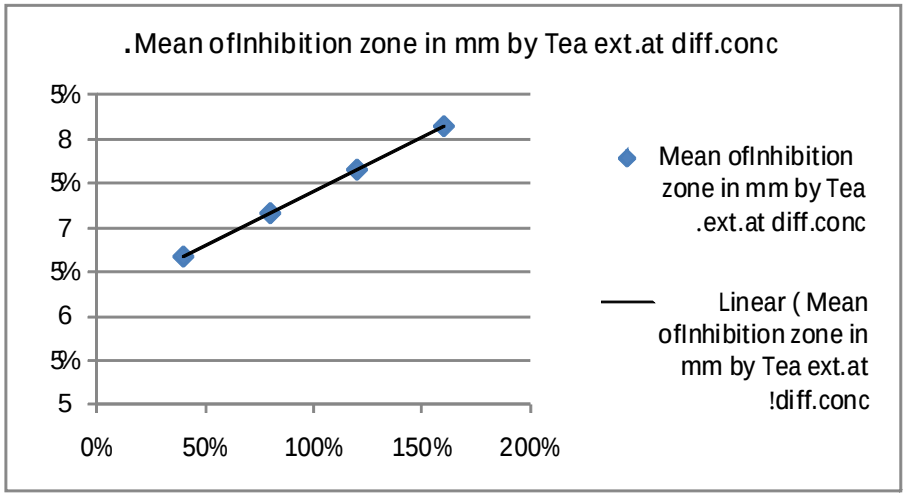

Figure 3. Correlation between different concentrations of tea extract and mean diameter of inhibition zone

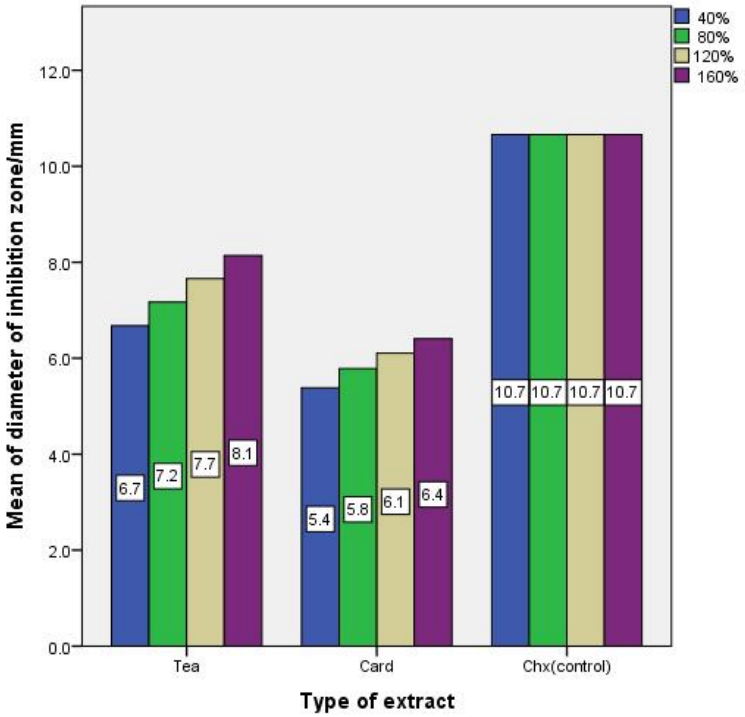

Figure 4: Mean diameter of inhibition zone by different concentrations of two types of extracts and control group.

Table 4: Mean count of MS by different concentrations for two types of extracts

Concentrations	Type of extract	N0.	Mean count of MS	±S.D.	t- test	P value
zero time	Tea	34	76.59	11.004	0.358	0.721
	Card	34	75.65	10.657		
40%	Tea	34	67.00	9.851	-1.684	0.09(NS)
	Card	34	70.50	9.740		
60%	Tea	34	61.53	8.999	-2.587	0.012
	Card	34	67.29	9.373		
120%	Tea	34	58.00	9.036	-2.587	0.002
	Card	34	64.74	9.571		
160%	Tea	34	52.68	9.550	-3.365	0.001
	Card	34	60.32	9.187		

Table 5: Mean count of MS in different concentration for three types of extracts

Concentrations/extracts		NO.	Mean of viable count of MS	±S.D.	95% Confidence Interval for Mean		F test	P value
					Lower Bound	Upper Bound		
zero time	Tea	34	76.59	11.004	72.75	80.43	128.465	HS
	Card	34	75.65	10.657	71.93	79.37		
	CHX	34	44.59	5.349	42.72	46.45		
40%	Tea	34	66.50	9.851	63.06	69.94	90.006	HS
	Card	34	70.50	9.740	67.10	73.90		
	CHX	34	44.59	5.349	42.72	46.45		
60%	Tea	34	61.53	8.999	58.39	64.67	71.961	HS
	Card	34	67.29	9.373	64.02	70.56		
	CHX	34	44.59	5.349	42.72	46.45		
120%	Tea	34	57.56	9.036	54.41	60.71	52.691	HS
	Card	34	64.74	9.571	61.40	68.07		
	CHX	34	44.59	5.349	42.72	46.45		
160%	Tea	34	52.68	9.550	49.34	56.01	30.925	HS
	Card	34	60.32	9.187	57.12	63.53		
	CHX	34	44.59	5.349	42.72	46.45		

Table 6. Comparison of mean viable count between two extract types and control group

Concentrations	(I) Type of extract	(J)Type of extract (CONTROL)	Mean Difference in mean of viable count of MS (I-J)	P value.	95% Confidence Interval	
					Lower Bound	Upper Bound
zero time	Tea	CHX	32.000*	.001	26.90	37.10
	Card	CHX	31.059*	.001	25.96	36.16
40%	Tea	CHX	21.912*	.001	17.25	26.58
	Card	CHX	25.912*	.001	21.25	30.58
60%	Tea	CHX	16.941*	.001	12.53	21.36
	Card	CHX	22.706*	.001	18.29	27.12
120%	Tea	CHX	12.971*	.001	8.51	17.44
	Card	CHX	20.147*	.001	15.68	24.61
160%	Tea	CHX	8.088*	.001	3.60	12.58
	Card	CHX	15.735*	.001	11.24	20.23

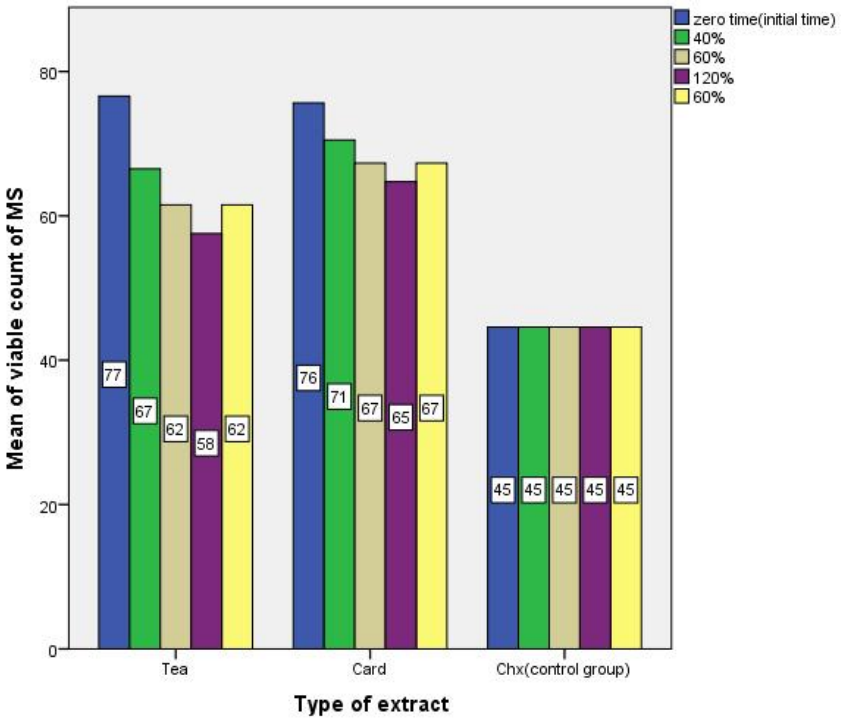

Figure 5. Mean of viable count of MS by different concentrations of two types of extracts and control group