

## The Effect of Green Tea on Prevention of Mouth Bacterial Infection, Halitosis, and Plaque Formation on Teeth

Abdolhosein Moghbel<sup>1\*</sup>, Ahmad Farjzadeh<sup>2</sup>, Nasrin Aghel<sup>3</sup>, Homayoon Agheli<sup>4</sup>, Nafiseh Raisi<sup>5</sup>

Received:29.4.2011

Accepted:21.5.2011

### ABSTRACT

**Background:** Compounds present in both green and black teas have been shown to inhibit the growth and activity of bacteria associated with mouth infections. The aim of this study was to assess the effects of green tea leaves extract on the aerobic mouth bacterial load.

**Methods:** A total of 25 volunteer female students, aged 20-25 years, were selected and then evaluated by green tea extract and mouthwashes containing 0.2, 0.5, and 1% tannin, as the most effective antibacterial complex in green tea. Then a comparative study was conducted on a green tea mouthwash containing 1% tannin with 10% ethanol, a alcohol free mouthwash, and a green tea herbal mouthwash with a chlorhexidine 0.2% sample, as and chemical brand.

**Results:** Green tea mouthwash containing 1% tannin was more effective than other concentrations ( $P<0.05$ ). There were no meaningful differences between the green tea mouthwashes containing 10% alcohol and alcohol free, as well as the herbal and chemical chlorhexidine 0.2% ( $P>0.05$ ).

**Conclusion:** The herbal green tea mouthwash could reduce the aerobic mouth bacterial load and may prevent plaque formation on teeth and come over halitosis due to infection of the bacteria. Also, it is a safe and nontoxic mouthwash especially for children and pregnant women.

**Keywords:** Bacterial Infection, Green Tea, Halitosis, Mouthwash, Plaque Formation, Teeth.

### INTRODUCTION

Plant extracts have been widely used in topical and oral applications for disease treatment. Examples of these include ginkgo biloba, echinacea, ginseng, grape seed, green tea, lemon, lavender, rosmmary, thuja, sara, allantoin, fever wort, blood root, apache plume, papaya, and tragacanth (1). Black tea is the second most commonly drunk liquid on earth after water. Green tea *Camellia sinensis* (Figure 1), which is not fermented at all during the drying

process, has numerous medicinal benefits mainly due to its antibacterial and antioxidant properties (2). Tea was essentially native to China but then it was transferred to India and Japan. Later on, it gained popularity in Europe and Russia, and, finally, in Iran.

A short list of phenolic phytochemicals with promising properties to benefit human health includes a group of polyphenol compounds called catechins (3-5) which are found in green tea (Figure 2).

1-Professor, Department of Pharmaceutics, School of Pharmacy, Jundishapur University of Medical Sciences, Ahvaz, Iran.

2- Associate Professor, Department of Microbiology, School of Medicine, Jundishapur University of Medical Sciences, Ahvaz, Iran.

3- Associate professor, Department of Pharmacognozy, Jundishapur University of Medical Sciences, Ahvaz, Iran.

4- Associate Professor, Department of Medicinal Chemistry, School of Pharmacy, Jundishapur University of Medical Sciences, Ahvaz, Iran.

5- Pharm D, Department of Pharmaceutics, School of Pharmacy, Jundishapur University of Medical Sciences, Ahvaz, Iran.

\*Corresponding author Email: drmoghbel@yahoo.com



Figure1. Fresh leaves of green tea

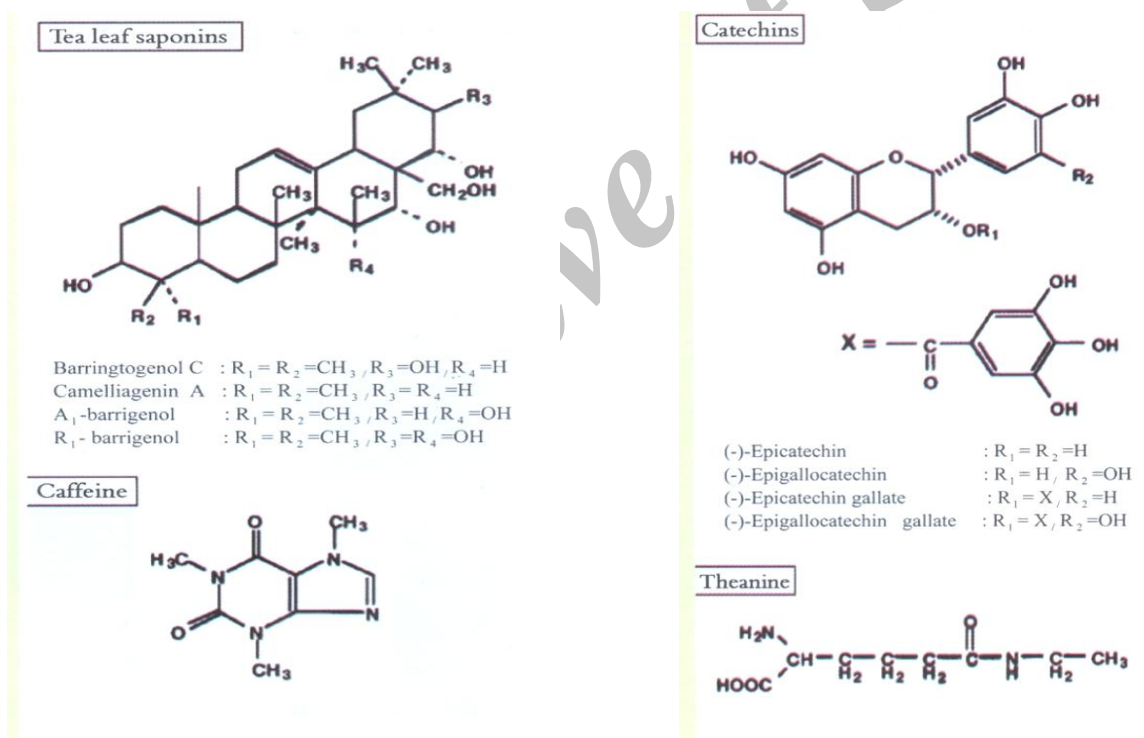


Figure 2: Chemical compound of green tea

Compounds present in both green and black teas have been shown to inhibit the growth and activity of bacteria with tooth decay producing halitosis (6,7). Animals and humans that have tea compounds in their drinking water develop fewer dental caries and less plaque formation than those drinking plain

water (8-11). Tooth decay is the gradual breakdown of the tooth beginning with the enamel surface and eventually progressing to the inner pulp. Tooth decay and, eventually, halitosis are caused by acids produced by certain mouth bacteria in dental plaque. Factors that affect this process include oral hygiene,

diet meal frequency, saliva production, and heredity. Teeth with significant decay are said to have caries or cavities (6,12-13).

The malefic bacteria in our mouths can cause tooth decay and bad breath (halitosis). Plaque is another contributor to bad breath. Pace University recently did a study on how effective green tea is in getting rid of bad breath. In this research project, they mixed green tea with the bacteria that cause tooth decay and strep throat. Results showed that green tea inhibited the bacterial growth by 30% and decreased the amount of bad breath producing compounds. Drinking green tea may also help prevent getting sore throats and colds since it helps fight the bacteria harboring in the throat (14).

Bad breath is caused by anaerobic sulfur producing bacteria that normally live on the surface of the tongue and in the throat. These bacteria are supposed to be there because they assist humans in digestion by breaking down the proteins found in specific foods, mucous, phlegm, blood, and diseased or "broken-down" oral tissues. Under certain conditions, these bacteria start to break proteins down at a very high rate. Proteins are made up of amino acids; two amino acids, cysteine and methionine, are dense with sulfur. When these "beneficial" bacteria come into contact with these compounds, the odorous and "lousy-tasting" sulfur compounds are released from the back of the tongue and throat as hydrogen sulfide, methyl mercaptan, and other odorous and bad tasting compounds. These "problem" compounds are often referred to as volatile sulfur compounds (14). Some reasons motivated us to examine the Iranian green tea antibacterial effect in order to use it later on as a mouthwash for blocking halitosis. As a matter of fact, we have been working on green tea for its healing effect on burns and wounds (15-16). In addition, it has been a traditional belief in Iran that when you have a bad breath due to alcoholic beverages, garlicky foods, or any other compounds which cause bad breath after digestion, you may chew some dry tea powder to block it. Based on one article, the phenolic compound in green tea may block the growth of bacteria responsible for teeth cavity and plaque formation (14). Therefore, the aim of this study was to evaluate the potency of

different extract concentrations of Iranian green tea as a mouthwash on mouth microbial load and then approving its effect on preventing halitosis or plaque formation in a separate study.

## MATERIALS AND METHODS

### *Leaves drying method and preparation of dried extract powder*

After the fresh leaves were picked from fields in Lahijan, a city in the north of Iran, they were spread out in hot air to wither. Once they became soft and pliable, they were traditionally pan-fried in woks, and after scientific evaluation by the Pharmacognosy Department, a voucher specimen was deposited at the herbarium of the faculty. In the final step, the leaves were dried by firing whereby, the natural fragrances and flavors were stabilized so that the leaves preserve their green color. The dried leaves were powdered mechanically and 200g of the 18 mesh powder was extracted by 1000 ml of deionized water at 70-80°C for 30 minutes. The extract was filtered by a cloth filter and concentrated under low pressure conditions with the device installed in a laminar airflow (FARPAJOO-Fee 2120-2). The extract was finally lyophilized (-40°C, 0.03 Tor) by a freeze-dryer (ZIRBUS Za co-5, Germany) for about 6 hours (6,17).

### *Tannin assay, validation, and preparation of calibration curve*

Stock solutions of standard tannic acid were prepared inside eleven 100 ml volumetric vessels each reagent containing zero to 10 ml of standard tannic acid, respectively. To each vessel, 1.5 ml of Folin-Denise reagent, 10ml of sodium carbonate solution, and sufficient deionized water were added to make the 100ml solution. Then, each sample was mixed vigorously until a light to dark blue color gradually appeared. The first vessel (free tannic acid) with no color was used as a blank sample. In order to complete the color reaction, all samples were held at room temperature for 3 hours. This process was repeated for three to six consecutive days to find the inter- and intra-day variations. Absorbance levels were measured spectrophotometrically (Shimadzu uv/visible spectrophotometer 2100; Toko, Japan) at 760 nm. The mean data (n=10) were

used for the preparation of the calibration curve (6,18). The tannin concentration for each sample (mg/100 ml) was calculated from the regression equation obtained from the calibration curve.

#### **Preparation of different equivalent tannin concentrations from the green tea powder extract**

Samples containing various concentrations of tannin (0.2, 0.5, and 1%) were prepared by equivalent amounts of 3.2, 8.02, and 16.05 mg of the dried green tea powder yielded from the 100mg dried extract, respectively (18). Each sample was deposited inside a 100 ml stoppered bottle which had already been sterilized by an autoclave at 121°C, 15 lb pressure for 20 minutes. The volume of each sample was adjusted at 100 ml with deionized water which was finally filtered through a 0.45 membrane filter to be sterilized.

#### **Antimicrobial evaluation of green tea extracts containing 0.2, 0.5, and 1 % tannin**

The participants, 25 female students aged between 20-25 years, were asked to wash their mouths with 10-15 ml deionized water for one minute at 12 p.m., and after brushing their teeth before 10 a.m. Mouth containing of each student was poured into sterile containers. Aliquots of 0.01 ml of each sample was diluted in 1ml deionized water under sterilization condition. Then an aliquot of each new sample was plated on petri dishes containing bloody agar. The plates were incubated inside a candle jar with 5-7 CO<sub>2</sub> concentration at 35-37°C for 24 hours and, then, colonies were counted separately (19). This process was repeated for the extracts containing 0.2, 0.5, and 1% tannin with the time interval of at least one day for each concentration before the trial with deionized water and then, the green tea extract.

#### **Preparation of mouthwash formulation No.1 (green tea mouthwash containing 1% tannin)**

A quantity of 16.5 mg green tea dried extract equivalent to 1% tannin was dissolved in about 70 ml of 50°C deionized water with 20 mg sodium saccharin as a non-sugar sweetener. The final volume was adjusted to 100 ml by deionized water after thorough mixing and paper filtering. The microbial activity of this

formulation was evaluated as the method mentioned above for the extract.

#### **Preparation of mouthwash formulation No.2 (green tea mouthwash containing 1% tannin with 10% alcohol)**

All evaluations on this formulation were performed the same as those on No.1 formulation. In this formulation, the role of 10% alcohol, which had been incorporated along with green tea, was evaluated on the microorganism.

#### **Comparison of the herbal green tea mouthwash with chemical chlorhexidine 0.2%**

In this evaluation, the formulation No. 2 (with 10% alcohol) was compared with a commercial brand known as chlorhexidine 0.2% on the mouth microbial content of the same students on separate tests.

#### **Coagulase test**

This test was run to identify *Staphylococcus aureus* species in a restrictive laboratory medium. *Staphylococcus aureus* cultures contain a coagulase enzyme which can coagulate blood plasma by action on fibrinogen. This enzyme does not exist in other staphylococcus which are known as negative staphylococcus. In this test, for preparation of the sample, one drop of normal saline was deposited on one side of a clean glass plate and with the culture beam a cycle of full microbial culture was dispersed in the normal saline drop. On the other side of the glass plate, one cycle full of microbial culture was deposited to make an emulsion after mixing. The mixture was agitated for about 3 minutes and when the plasma began to dry, one drop of saline was added. In this evaluation, if coagulase culture were positive, a granule mode would appear. This mode would not be seen in the mixture of culture in saline (control); therefore, in the case of negative result, no granule residue would appear (19).

#### **Oxidase test**

This test was done for identifying bacteria suspected to *Neisseria*. The tetramethylparaphenyl aminodehydrochloride 0.1% solution as the reagent was prepared with deionized water. A filter paper was installed in a clean plate and then few drops of the reagent

were added to the surface of the filter. Finally, using a platinum loop, few colonies of the bacterium were spread on the wet side of the paper filter so that in case of positive result for live bacteria, an intensive purple color would appear after 10 seconds (19).

#### Statistical analysis and validation

Statistical comparisons were carried out by t-test and one-way analysis of variance (ANOVA) for paired and also independent samples using SPSS software ( $P < 0.05$ ). The results were expressed as mean  $\pm$  SD. The inter

and intra- day variations data for validation of the calibration curve were applied, and the concentration of the equivalent dissolved drug was calculated from the regression equation obtained from the standard deviation.

#### RESULTS

The inter- and intra-day variations of standard deviation and accuracy of the tannic acid assay (the most important active material) are shown in Tables 1 and 2.

**Table 1.** The inter-day variation data of the standard curve and accuracy results for tannic acid assay by Folin- Denis method

Concentration mg/100 ml	Absorbance Mean (n=3) $\pm$ SD	CV%	Accuracy% Mean $\pm$ SD
0.1	0.066 $\pm$ 0.005	8.66	91.57 $\pm$ 8.81
0.2	0.163 $\pm$ 0.005	3.53	114.33 $\pm$ 4.09
0.3	0.193 $\pm$ 0.005	2.98	90.40 $\pm$ 2.72
0.4	0.29 $\pm$ 0.00	0	102.07 $\pm$ 0.00
0.5	0.343 $\pm$ 0.011	3.36	96.79 $\pm$ 3.27
0.6	0.423 $\pm$ 0.0152	3.60	99.56 $\pm$ 3.61
0.7	0.493 $\pm$ 0.011	2.34	99.52 $\pm$ 2.33
0.8	0.573 $\pm$ 0.005	1.00	101.26 $\pm$ 1.02
0.9	0.63 $\pm$ 0.00	0	98.94 $\pm$ 0.00
1	0.706 $\pm$ 0.005	0.81	99.92 $\pm$ 0.81

**Table 2:** The inter-day variation data of the standard curve and accuracy results in 6 different days and triplicate for tannic acid absorbance and assay by Folin –Denis method.

Concentration mg/100 ml	Absorbance Mean (n=3) $\pm$ SD	CV%	Accuracy% Mean $\pm$ SD
0.1	0.066 $\pm$ 0.002	3.2	91.57 $\pm$ 2.98
0.2	0.175 $\pm$ 0.004	2.7	110.39 $\pm$ 3.55
0.3	0.195 $\pm$ 0.002	1.7	91.71 $\pm$ 1.54
0.4	0.291 $\pm$ 0.001	0.5	103.25 $\pm$ 2.04
0.5	0.353 $\pm$ 0.006	1.8	100.40 $\pm$ 2.7
0.6	0.421 $\pm$ 0.002	0.6	98.91 $\pm$ 0.77
0.7	0.500 $\pm$ 0.004	0.9	101.55 $\pm$ 1.01
0.8	0.574 $\pm$ 0.001	0.2	101.46 $\pm$ 0.30
0.9	0.626 $\pm$ 0.005	0.5	98.68 $\pm$ 0.55
1	0.706 $\pm$ 0.001	0.9	99.84 $\pm$ 0.19

The result of mean ± SD for the assay of tannin in green tea extract by Folin-Denis method repeated in three consecutive days in

triplicate was 6.23 ± 0.19 mg of tannin in 100 mg of the dried powder extract of green tea ( Figure 3, Table 3).

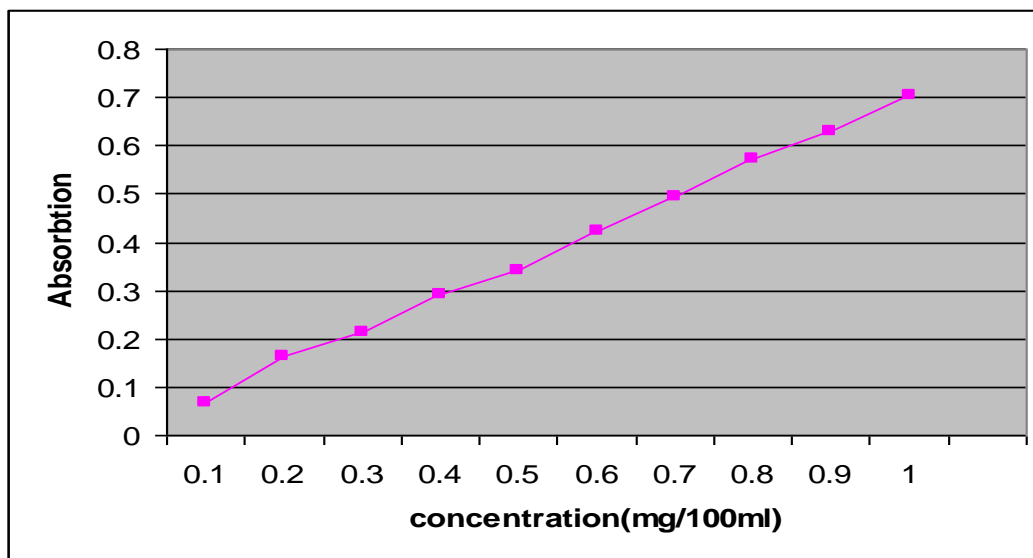


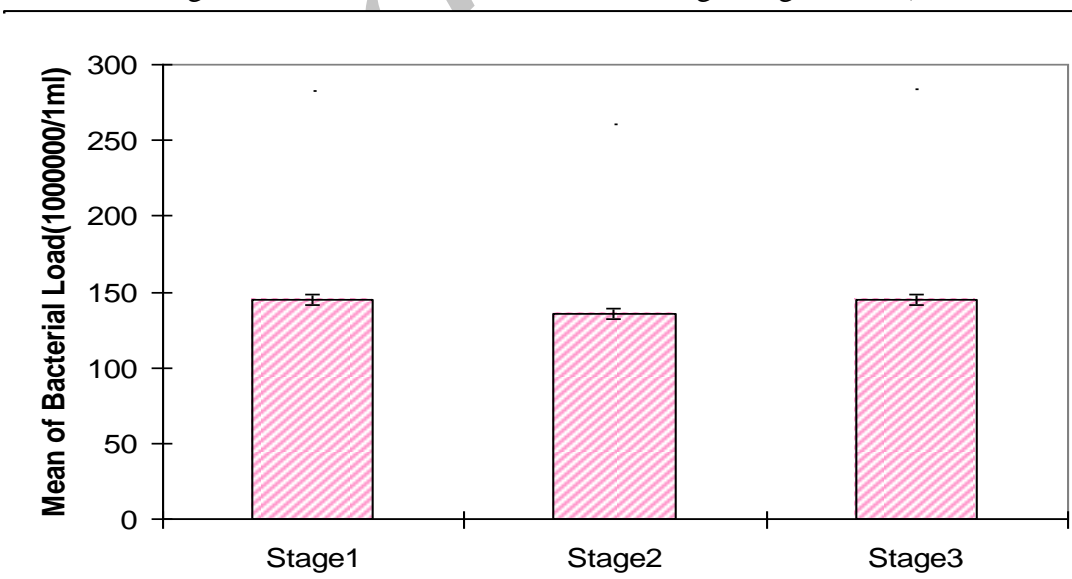
Figure 3: Standard curve of tannic acid

Table 3. Tannin assay result of green tea leaves (n=3) Equivalent amount of tannin concentration

Sample No.	Weight of green tea extract, total (mg)	Absorbance	Tannin concentration Mg/100ml	Tannin % in total extract
1	14.28	0.61	0.86	6.03
2	14.28	0.63	0.89	6.23
3	14.28	0.65	0.91	6.37
Mean ± SD	14.28	0.63±0.02	0.88±0.025	6.23±0.19

The effectiveness of water as a negative control or drug free mouthwash in different

stages of mouth washing (1 and 2 hours after beginning the trial ) are shown in Figure 4



Different time stages

Zero

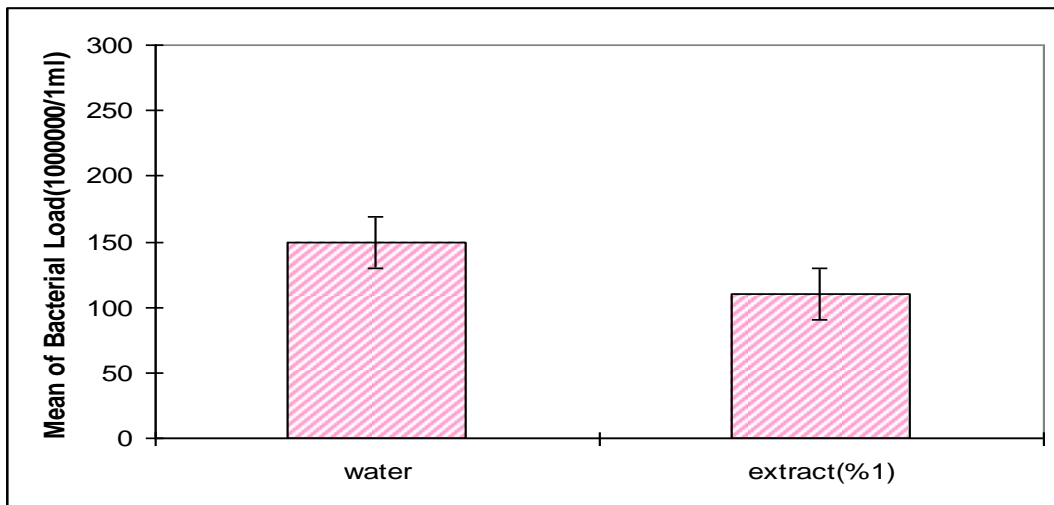
One hour

Two hours

Figure 4: The effect of water on mean of mouth bacterial load at different times

The comparative results between water as a negative or drug free control and different concentrations of tannin (0.2 ,0.5, and 1% ) as

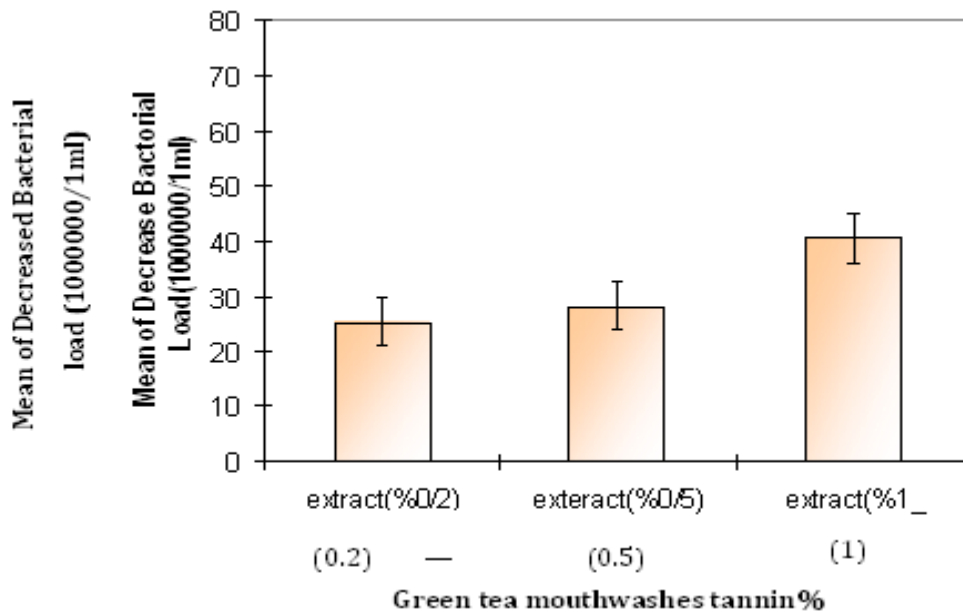
the most effective antibacterial agent in green tea leaves are demonstrated in Figure 5 (0.2 and 0.5% not shown).



**Figure5:** The comparative effect of tannin 1% extract and simple water on the mean of mouth bacterial load.

The results for different concentrations of 0.2, 0.5, and 1% tannin samples showed about

16, 17.5 and 26% reductions on the aerobic mouth bacterial load, respectively (Figure 6).



**Figure6:** Comparing three different concentrations of green tea tannin on the reduction of aerobic mouth bacterial load.

The comparative result of the effect of the mouthwashes containing 1% tannin along with 10% alcohol and free-alcohol showed

32% bacterial reduction for the alcoholic mouthwash and 26% reduction for the free-alcohol mouthwash (Figure7).



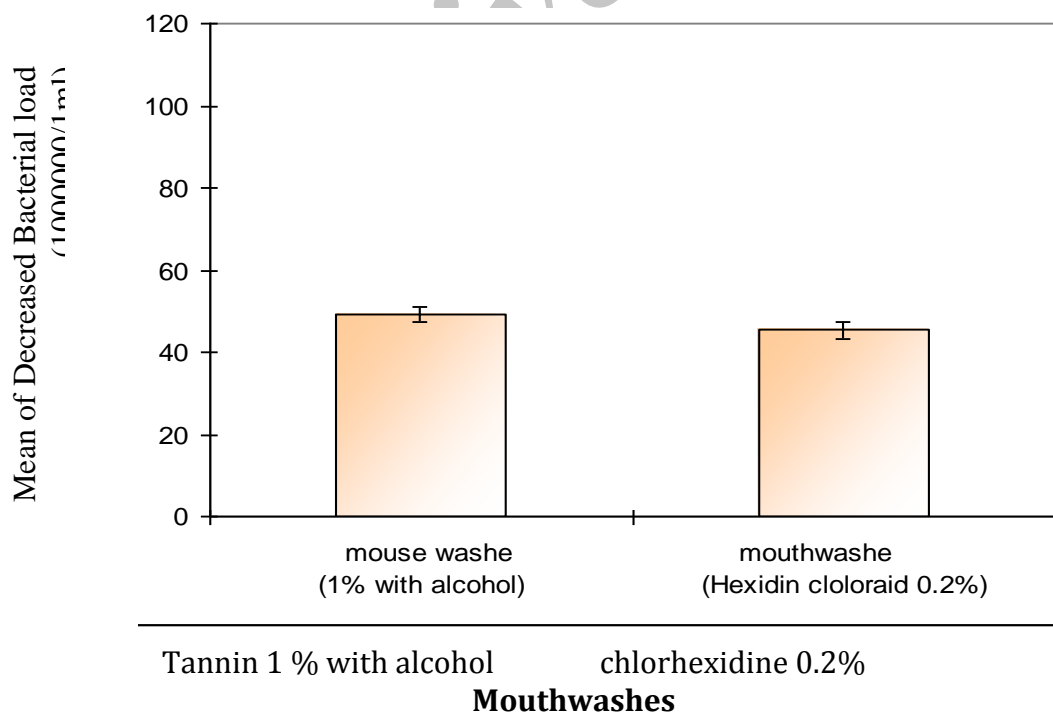
**Green tea tannin 1% mouthwashes**

**Figure 7:** Green tea effect of mouthwashes containing tannin 1% with and without alcohol on the reduction of aerobic mouth bacterial load.

**Green tea tannin 1% mouthwashes**

Evaluation of the effectiveness of the 1% tannin green tea in comparison with the chemical chlorhexidine 0.2% mouthwash

showed that both mouthwashes induced the same reduction (about 32%) in the bacteria (Figure 8).



**Figure 8:** Comparing mouthwashes effect of green tea containing alcoholic tannin 1% with chlorhexidine 0.2% on the reduction of aerobic mouth bacterial load.



The results of coagulase and oxidase tests showed that no granule was formed; therefore, the coagulase test was negative; however, the oxidase test was positive because of the intensive purple color which was evidence of *Neisseria* in the presence of idophenoloxidos enzyme (19).

## DISCUSSION

Application of an appropriate mouthwash has the following benefits: having antiseptic effects on mouth, washing the food residue on the gingiva (gum) medium and teeth, reducing the mouth bacteria, masking and neutralizing halitosis, and introducing a good taste and sense of freshness, having anti-plaque, anti-stain, and anti-tartar properties, acting as a fluoride depot, preventing tooth cavities that help wound healing and gum injuries, decreasing the incidence of tooth rash and herpes, preventing bad breath, producing a fresh mint taste, helping the health of milk teeth, and protecting the permanent teeth of children and adults (12,13). In a broad field of medicine, several researchers have published various articles on the effects of green tea, such as reducing and adjusting the weight (20), curing dermal inflammation and disorders (21,22), healing wounds (15,16), preventing immature and ill time of human skin (23), preventing and curing some cancers, reducing blood pressure, cholesterol, and sugar, preventing heart diseases, and adjusting hormones which promote health and long life (24-29). One effect that no article has pointed out so far is the probability of showing dermal anti-sensitivity effects on bringing itching and urticaria to a stop. Also, a recent article introduced antibacterial and anti-caries effects for green tea (30). The main purpose of this study was to test this fact, in lab condition and also, clinically. Green tea contains flavonoids, tannin, vitamins, fluoride, and other mineral salts. Some antioxidant and antimicrobial agents of green tea could increase the life and efficiency of teeth (31-33). Tannins are biosynthesis materials which have a potent antibacterial effect. In recent decay researches, it has been shown that green and black teas prevent installation of bacteria and, therefore, result in the formation of plaques on teeth. This results in the decreasing construction of human

amylase excretion, preventing glucosyl transferase and, finally, limiting glucan biosynthesis which gets stuck on teeth. In addition, it was specified that the routine consumption of green tea in human studies could reduce the intensity of teeth caries (34-36). The results of mouth washing with different tannin concentrations of green tea for the participants showed a different reduction in bacterial colonies. For example, the tannin 1% green tea mouthwash caused a better and more significant reduction in bacteria (26%) compared with 0.5 and 0.2 concentrations ( $P < 0.05$ ). The findings of the study by Yamamoto *et al.* on green tea in the United States of America in 1997 showed that green tea extract containing 0.5% tannin with 2% alcohol decreased the different types of staphylococcus about 15% (37). However, in Iranian alcohol free tannin 0.5% green tea mouthwash, there was a 16% reduction in the bacteria. This shows that Iranian green tea may contain a higher amount of tannin. The yield value of tannin in 100 mg extract powder of Iranian green tea was calculated to be  $6.23 \pm 19$  mg. The assay of tannin was performed in triplicate with a mean CV-percentage of about 2.6 and accuracy percentage of  $100 \pm 3.33$ . This result indicates that Folin-Denis method can be accepted as a specific method for this assay. Another significant point in comparing Yamamoto's research with this study is the difference in choosing the amount of tannin in the formula (37). This is an important factor which may affect the intensity of microbial death. Applying a sample with 1% tannin instead of 0.5% could increase the microbial death as much as two-fold (26%) and even more (15% - 32%) when 10% alcohol is charged. This arrangement of the formulation showed its preference and also the better idea of the formula. Although the mouthwash with 10% alcohol had a result of 6% more in reduction of mouth bacteria, this increase was not significant enough ( $P > 0.05$ ). Most pharmaceutical references have reported a range of 15-20% for the preservative effect of alcohol (ethanol) in pharmaceutical dosage forms and also 60-70% as an antibacterial or antiseptic dermal solution (36-38). Having in mind the above mentioned fact about alcohol its side effects and benefits, depending on the

person who is using the mouthwash (adults, children, or pregnant women) and when it is used, during breast feeding or other times, green tea may be used with 10% alcohol or just as alcohol free green tea. Therefore, we decided not to omit alcohol from the final formulation but emphasize that children, pregnant women, and mothers who are at the breast feeding period should use the free-alcohol type.

In this study, a comparison was made between green tea extract and water as a negative control to make sure that water did not influence the death or removal of the bacteria during rinsing out the mouth with water. The results of mouth rinsing with water at different times (e.g. 0, 1, and 2 hours) which were done to understand the time interval of the bacteria return to the mouth showed a significant difference between the loads of bacteria in mouth one and two hours after rinsing ( $P < 0.05$ ). There were no differences between stages one and three ( $P > 0.05$ ). Therefore, the time interval for the bacteria to come back to the same amount of the starting time was indicated to be about 2 hours (Figure 4). Hence, the reason of choosing this lag time between the evaluation of different concentrations of tannin and the stages of brushing or washing the mouth with water 2 hours before testing the mouthwashes was as mentioned above. The comparison of Figures 2 and 4 showed a clearly significant difference between all concentrations of the green tea extracts (0.2, 0.5, and 1% tannin) and water as negative control ( $P < 0.05$ ). This could be estimated from the 17-34% decrease in the mean bacterial load of mouth. However, there were no significant differences between the results of 0.2 and 0.5% tannin extract ( $P > 0.05$ ). In addition, the comparison of the mouthwash containing 1% tannin with water showed the same significant difference as tannin extract alone ( $P < 0.05$ ), but the mean decrease in the bacterial load of the mouthwashes containing 1% tannin with and without alcohol (Figure 7) had small but not significant differences ( $P > 0.05$ ). The comparison of chlorhexidine 0.2% with water (Figure not shown) also showed a significant difference ( $P < 0.05$ ) but the difference in mean decrease for bacterial load between tannin 1% containing alcohol

with chlorhexidine 0.2% (Figure 8) was not significant ( $P > 0.05$ ). However, it is not necessary for alcohol to be formulated with tannin extract in green tea mouthwash chemically and, moreover, toxicologically because of being safe for children and pregnant women or during breast feeding. However, it is possible and somehow preferable to use 10% alcohol in the formula as a protective or preservative agent during use at home and opening the door of the container several times. Of course, adding alcohol to the mouthwash, despite being harmful for children or others, may have some benefits in the process of formulation. For example, acting as a co-solvent, especially in the case of oily materials (essential oils), promoting the expiry and stability date, refreshing the taste contraction and, therefore, severity of the gum and tongue, and, eventually, helping the sense of taste and teeth whitening. The results of coagulase and oxidase tests on culture medium showed that two kinds of bacteria showed better reductions. Purification of the sample and identification of the bacteria resulted in the negative coagulase and species of *Neisseria*. It should be notified that limited identification of these bacteria of mouth only by above tests is not sufficient so biochemical tests and even the use of molecular biologic tests based on ribosomal PCR and RNA would be more valid. In case of having a safe and nontoxic mouthwash for human, allergic signs, such as throat, mouth, and tongue mucous burn, red surface and irritation of lips, gum, and tear glands, should not leak out. The prickly and heat sense in mouth due to using mouthwashes containing some alcohol is normal because of the contraction between alcohol molecules and saliva which is also tolerable. To wash away the normal needed fluoride and saliva enzyme along with pathogen microorganism of mouth presents difficulties with some mouthwashes by chelating with chemical ions. In addition, the probability of staining the teeth by some chemical mouthwashes should be considered. The reason of this problem could be the trend of change of pH and, therefore, the stability of chemical mouth washes because of having reactive elements and materials. To make sure that the stability of the mouthwash is constant after formulation, the evaluation of pH for

green tea mouthwash must not have significant changes in pH in three stages ( $P > 0.05$ ) (Table 4).

**Table 4.** The result of pH changing after formulation (Mean  $\pm$  SD)

Evaluation time after formulation	Mean of pH $\pm$ SD
48 hours	7.63 $\pm$ 0.04
One week	7.62 $\pm$ 0.03
One month	7.58 $\pm$ 0.026
Three months	7.60 $\pm$ 0.043

Furthermore, the study of chemical stability for green tea mouthwash was followed by calculating tannin remaining unchanged during zero to 90 days 3 times

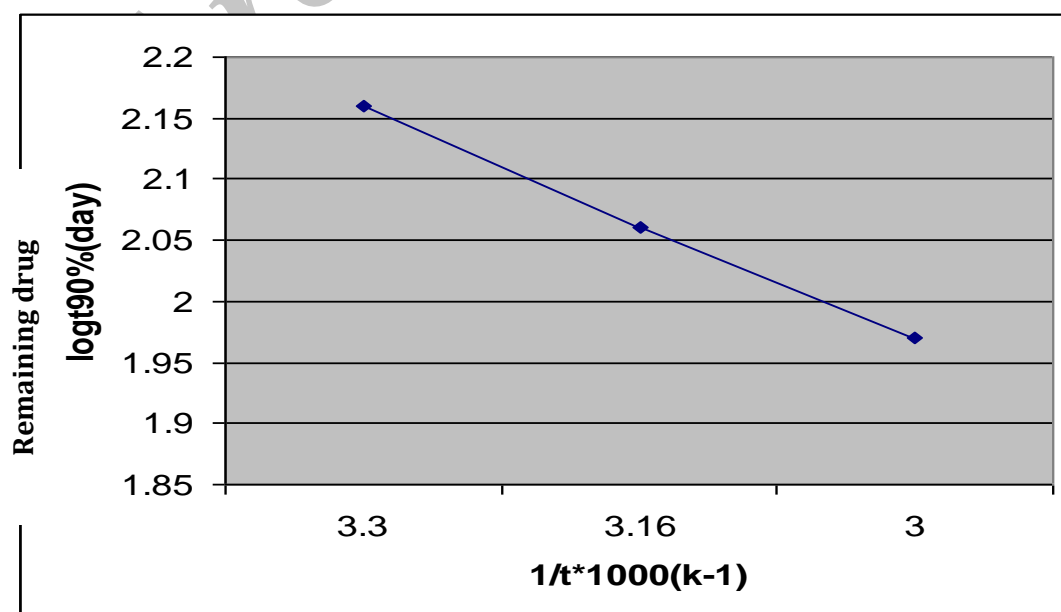
after holding the mouthwash at 30, 45, and 60 °C temperatures (38) in separate tests (Tables 5&6 and Figures 9&10).

**Table 5:** Tannin % remaining unchanged during zero to 90 days of different temperatures

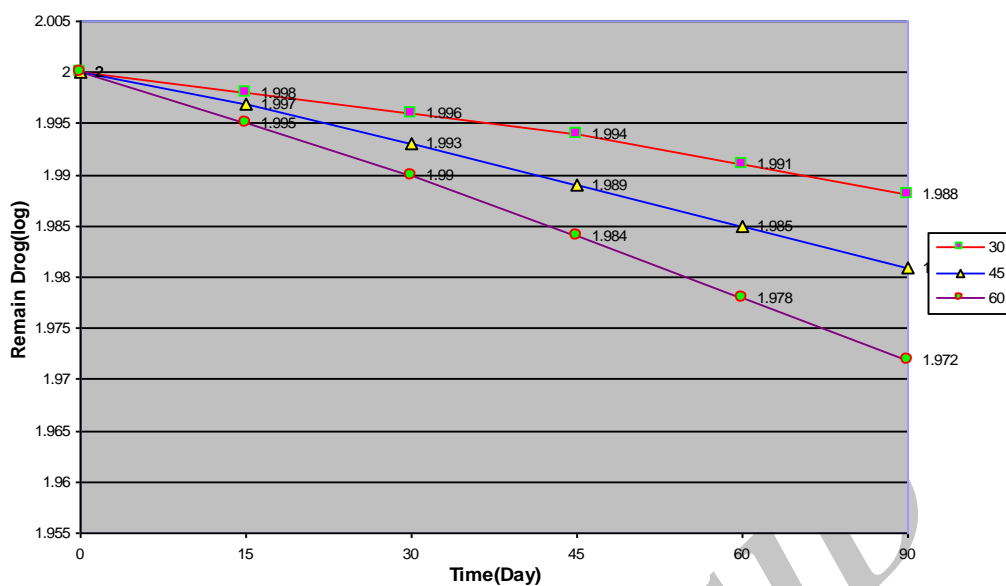
Time (days)	Remaining tannin (%) in different temperatures		
	30 °C	45 °C	60 °C
0	100	100	100
15	99.7 $\pm$ 0.531	98.47 $\pm$ 0.921	99.09 $\pm$ 0.531
30	99.4 $\pm$ 0.00	98.47 $\pm$ 0.921	97.86 $\pm$ 0.531
45	98.78 $\pm$ 0.531	69.94 $\pm$ 0.531	96.63 $\pm$ 0.921
60	98.86 $\pm$ 0.531	69.94 $\pm$ 0.531	94.79 $\pm$ 0.00
90	96.63 $\pm$ 0.00	94.79 $\pm$ 0.921	92.03 $\pm$ 0.921

**Table 6:** Linear parameters data of figure 10

Linear specifications	Temperature (°C)		
	30	45	60
Correlation coefficient	0.9679	0.9874	0.9901
Regression coefficient	0.0002	0.0003	0.0004
y-intercept	2.0013	2.007	2.0018



**Figure 9.** The log of remaining drug versus time.



**Figure10.** Log  $t_{90}\%$  curve versus of the reciprocal Kelvin temperature.

Generally, any dosage form which has its active drug as much as 90% of the initial dose of the formulation is suggested to be stable and legally authorized to be used (38). Studies have shown that tea can eliminate bad breath (halitosis). Green tea helps toothpaste and mouthwashes fight viruses by eliminating the bacteria. It also helps remove plaque built up within gums and teeth which is another contributor to bad breath. Therefore, green tea may combat bad breath by drinking it in large quantities, using it as a mouthwash before and after tooth brushing, or mixing it with toothpaste before tooth brushing.

### Conclusion

A herbal mouthwash formulation of Iranian green tea extract containing 1% tannin could reduce the aerobic mouth bacterial load about 26-32% and also, due to this reduction it may prevent plaque formation on teeth and therefore, halitosis. These two need to be approved by more study later. Depending who is using the mouthwash, adults, child or pregnant women and, when it is using, during breast feeding or the other times, green tea with 10% alcohol or alcohol free green tea may be used. But children, pregnant women and mothers who are at breast feeding period should use the alcohol free mouthwash.

### Acknowledgements

This paper is issued from thesis of Nafiseh Raisi, Pharm.D and financial support was provided by Ahvaz Jundishapur University of Medical Sciences.

### REFERTENCES

1. Moghbel A, Hemmati A, Agheli H, Amraee K, Rashidi I. The effect of tragacanth mucilage on the healing of full-thickness wound in rabbit. *Archives of Iranian Medicine*. 2005;8(4):257-62.
2. David W, Sifton R. PDR for herbal medicines. 4<sup>th</sup> ed: Thomason ; 2004 :408- 14.
3. Mukhtar H, Gupta H, Ahmad N. Inhibition of nuclear transcription factor NF $\kappa$ B by green tea constituent epigallocatechin 3- gallate in human epidermoid carcinoma cells A 431.J *dermatol Sci* 1998; 16:50-55.
4. Chopra D, Simon D. The Chopra Center Herbal Handbook. The Chopra Center Herbal Handbook. 2000.
5. Moghbel A, Abbaspoor H. A study on the factors affecting the compressibility of green tea leaves powder to make a herbal tablet. *SCIENTIFIC MEDICAL JOURNAL*. 2010;8(4):78-463.
6. Rasheed A, Haider M. Antibacterial activity of *Camellia sinensis* extracts against dental caries. *Archives of Pharmacal Research*. 1998;21(3):348-52.
7. Matsumoto M, Minami T, Sasaki H, Sobue S, Hamada S, Ooshima T. Inhibitory effects of oolong tea extract on caries-inducing properties of

- Mutans streptococci. Caries research. 2000;33(6):441-5.
8. Otake S, Makimura M, Kuroki T, Nishihara Y, Hirasawa M. Anticaries effects of polyphenolic compounds from Japanese green tea. Caries research. 1991;25(6):438-43.
  9. Ooshima T, Minami T, Aono W, Izumitani A, Sobue S, Fujiwara T, et al. Oolong Tea Polyphenols Inhibit Experimental Dental Caries in SPF Rats Infected with Mutatis Streptococci. Caries research. 1993;27(2):124-9.
  10. Ohshima T, Minami T, Matsumoto M, Fujiwara T, Sobue S, Hamada S. Comparison of the cariostatic effects between regimens to administer oolong tea polyphenols in SPF rats. Caries Res,1998; 32: 75-80.
  11. Ooshima T, Minami T, Aono W, et al. Reduction of dental plaque deposition in humans by oolong tea extract caries Res.1994;28:146-9.
  12. Lang Np, Lindhe J. Clinical periodontology and ampler dentistry. 5<sup>th</sup>ed, Iowa (USA) Blackwell; 2008 :183-202.
  13. Caranza F, Newman M. Clinical periodontology. 10<sup>th</sup> ed. Philadephia: WB saunders; 2006: 684-883.
  14. Blasingame J. Green tea prevents bad breath.[cited 2009]. Available from: <http://blog.therabreath.com/2009/08/green-tea-prevents-bad-breath>.
  15. Rahimzadeh F, Moghbel A Kalantar A. formulation of wound healing cream from Iranian green tea extract. A thesis presented for the pharmacy doctorate degree, Ahvaz Jundishapur University of Medical Sciences , Ahvaz, Iran 2006 :81 -92 (unpublished ).
  16. Rahimzadeh F, Moghbel A. Apilot study of theraputic effect of water extract (infusion) of Iranian green tea on the healing of full-thickness wound in rabbit. No. 482 approved research project at research deputy of Ahvaz Jundishapur University of Medical Sciences Ahvaz, Iran 2004 :20-5 (unpublished).
  17. Tory DB. Remington: The science and practice of pharmacy 21<sup>th</sup>ed. Philadelphia, Lippincott William & Wilkins; 2005: 706- 1061.
  18. Waterman PG, Mole S. Analysis of phenolic plant metabolits 1<sup>th</sup>ed. Blackwell Scientific Publication; 1999:13-83.
  19. Baron EJ, Bailey WR, Finegold SM, Bailey & Scott's. Diagnostic Microbiology 8<sup>th</sup>ed. St. Louls: C.V. Mosby Company; 1990:72-95.
  20. Westerterp MS, Lejeune MP, Kovacs EM. Body weight loss and weigtght maintenance in relation to habitual caffeine intake and green tea supplementation. Obes Res ,2005; 13(7):1195-204.
  21. Hsu S. Green tea and the skin. Journal of the American Academy of Dermatology. 2005;52(6):1049-59.
  22. Vayalil PK, Elmets CA, Latiyar SK. Treatment of geent tea polyphenols in hydrophilic cream prevents UVB – induced oxidation of lipids and proteins depletion of antioxidant enzyme and phoshphorylation of MAPK proteins in SKH-1 hairless mouse skin. Carcinogensis, 2003; 24 (5):927- 36.
  23. Chiu AE, Chan JL, Kern DG, Kohler S, Rehmus WE, Kimball AB. Double - Blinded, Placebo - Controlled Trial of Green Tea Extracts in the Clinical and Histologic Appearance of Photoaging Skin. Dermatologic surgery. 2005;31:855-60.
  24. Fukino Y, Shimbo M, Aoki N, Okubo T, Iso H. Randomized controlled trial for an effect of green tea consumption on insulin resistance and inflammation markers. Journal of nutritional science and vitaminology. 2005;51(5):335-42.
  25. Choan E, Segal R, Jonker D, Malone S, Reaume N, Eapen L, et al., editors. A prospective clinical trial of green tea for hormone refractory prostate cancer: an evaluation of the complementary/alternative therapy approach2005: Elsevier.
  26. Seely D, Mills EJ, Wu P, Verma S, Guyatt GH. The effects of green tea consumption on incidence of breast cancer and recurrence of breast cancer: a systematic review and meta-analysis. Integrative cancer therapies. 2005;4(2):144.
  27. Suzuki Y, Tsubono Y, Nakaya N, Koizumi Y, Tsuji I. Green tea and the risk of breast cancer: pooled analysis of two prospective studies in Japan. British journal of cancer. 2004;90(7):1361-3.
  28. Ishikawa A, Kuriyama S, Tsubono Y, Fukao A, Takahashi H, Tachiya H, et al. Smoking, alcohol drinking, green tea consumption and the risk of esophageal cancer in Japanese men. Journal of epidemiology. 2006;16(5):185-92.
  29. Kuriyama S, Shimazu T, Ohmori K, Kikuchi N, Nakaya N, Nishino Y, et al. Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan. JAMA: the journal of the American Medical Association. 2006;296(10):1255.
  30. Wolinsky L, Cuomo J, Quesada K, Bato T, Camargo P. A comparative pilot study of the effects of a dentifrice containing green tea bioflavonoids, sanguinarine or triclosan on oral bacterial biofilm formation. The Journal of clinical dentistry. 2000;11(2):53.
  31. Bérubé-Parent S, Pelletier C, Doré J, Tremblay A. Effects of encapsulated green tea and Guarana extracts containing a mixture of epigallocatechin-3-gallate and caffeine on 24 h

energy expenditure and fat oxidation in men. *British Journal of Nutrition*. 2005;94(3):432.

32. Ferrara L, Montesano D, Senatore A. The distribution of minerals and flavonoids in the tea plant (*Camellia sinensis*)\* 1. *Il farmaco*. 2001;56(5-7):397-401.

33. du Toit R, Volstedt Y, Apostolides Z. Comparison of the antioxidant content of fruits, vegetables and teas measured as vitamin C equivalents. *Toxicology*. 2001;166(1-2):63-9.

34. Paye M, Barel AO, Maibach HI. *Handbook of cosmetic and technology*. 2<sup>th</sup> ed. New York. CRC press, 2006:98.

35. Serafini M, Ghiselli A, Ferro-Luzzi A. In vivo antioxidant effect of green and black tea in man. *European journal of clinical nutrition*. 1996;50(1):28.

36. Martindale, Sweetman SC, Martindale W. *The complete drug reference: Pharmaceutical Press* (2002). Martindale: *The complete drug reference, thirty-fifth edition*, London; 2005.

37. Yamamoto T. *Chemistry and applications of green tea: CRC*; 1997.

38. Banker G, Anderson N, Lachman L. *The theory and practice of industrial pharmacy. The theory and practice of industrial pharmacy*. 1986.

Archive of SID