Effects of Green Tea in Oral Bacteria

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Abstract: Compounds present in green tea have provided to inhibit the growth and activity of bacteria associated with infections. To assess the effects of green tea leaves extract in presence of propylene glycol on the aerobic mouth bacteria load. Saliva of 2 volunteer girl aging 20-23 years were taken and evaluated by a mouthwash sample containing 1% tannin, as the most effective antibacterial complex in green tea. Comparative studies were also conducted between green tea mouthwash containing 1% tannin and a similar sample with 10% propylene glycol added during extraction. This comparison was applied for a chlorhexidine 0.2% sample as a chemical mouthwash brand too. There was a meaningful difference between the green tea mouth washes containing 10% propylene glycol and the simple green tea extract (P<0.05). Significant difference was also seen between the herbal and chemical mouthwashes (P<0.05). The extract 1% tannin containing 10% propylene glycol reduced the aerobic mouth bacterial load of the student saliva about 64%. The pH monotonousness in the days and temperature approved the stability of tannin in liquid. Using green tea extract as a herbal mouthwash is safe and harmless specially for children and pregnant women. The result led us to suppose that green tea may prevent plaque formation on teeth, coming over halitosis due to mouth infection, too. These effects need to be approved in an in vivo trial as a second study. Plant extract have widely used as tropical and oral applications for disease treatment. Black tea is the second most commonly drank liquid on the earth after water. Green tea camellia sinensis which is not fermented at all during drying process has numerous medicinal benefits mainly due to its antibacterial and antioxidant properties. Tea is native to china but then spread India and Japan, then to Europe, Russia and finally Iran. A short list of phenolic phytochemicals with promising properties to benefit human health includes a group of polyphenol compounds called catechins, found in green tea. Compounds present in both green and oolong tea have been studied on dental caries and less plaque formation than those drinking plain water. Drinking green tea may also help prevent sore throats and colds, since it help fight the bacteria harboring in the throat, another reason that encouraged us to work on Iranian green tea other than its healing effects on burn and wound, which was also in the area of our studies was a traditional belief for its masking the bad breath. In its methodology it includes green tea leaves drying methods and after that its powder extraction. Fresh green tea leaves were spread out in the hot air to wither. Once they became soft and pliable, they were traditionally pan-fried in woks. In the final step, the leaves were dried by firing as far as the natural fragrances and flavours stabilized and the leaves kept their green colour. The dried leaves were powderd mechanically, to obtain the extract. After the preparation of green tea powder stock solution of standard tannic acid were prepared. This process was repeatet for three or six consecutive days to find the inter-day and intra-day variations. The abosorption level was measured spectrophotometrically. After this process preparation of tannin from green tea powder extract is done. Sample containing 1% tannin was prepared by equivalent amount of dried green tea powder yielded from 100mg dried extract. The sample was poured into a 100ml stoppered bottle which has been sterilized by an autoclave at 121 degree, 15h, pressure for 20min. After this process next is preparation of mouthwash formulation is done. A quantity of 16.5mg green tea dried extract equivalent to 1% tannin was dissolved in about 70ml, 50°C deionized water and 20mg sodium saccharin as a non-sweetener. This formula was also repeated with the extra 10% propylene glycol. The anti microbial activity of PG formulation was compared with the chemical chlorhexidine 0.2%. The specific aim of this study was to determine the influence of propylene glycol (PG) as a co-solvent, on the potency of green tea extract as a mouthwash, on the staphylococcus aurous strains and Neisseria species of the mouth microbial load. Also, to demonstrate a comparative assessment between the herbal green tea extract containing PG and a chemical brand mouthwash in decreasing mouth bacterial contamination.

Keywords: aerobic mouth bacterial load, antibacterial complex, catechins, extraction of green tea

1. Introduction

Green tea is a type of tea that is made from Camellia sinensis leaves that have not undergone the same withering and oxidation process used to make oolong teas and black teas. Green tea originated in China, but its production and manufacture has spread to many other countries in Asia.

Several varieties of green tea exist, which differ substantially based on the variety of C. sinensis used, growing conditions horticulture methods, production processing, and time of harvest.

Steeping, Brewing and Serving

Steeping or brewing is the process of making tea leaves and hot water. Steeping temperature range from 61°C (142°F) to 87°C (189°F) and steeping times from 30 seconds to three minutes.

Extracts

Green tea extracts have been used in traditional Chinese and Indian medicine for a variety of uses. Green tea leaves are initially processed by soaking in an alcohol solution, which may be further concentrated to various levels, by

Volume 6 Issue 6, June 2018

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products of the process are also packaged and used. Extracts may be solid in liquid, powder, capsules, or tablet form. Decaffeinated versions are also available. Green tea extract supplements are accessible over the counter in various forms. Standardized green tea extract is 90 % total polyphenols, and 1 capsules equals 5 cups of tea.

Epigallocatechin and caffeine. and 100mL Regular The Camellia polyphenols various extract. Extracts products. colour contains Decaffeinated forms. supplements may green be supplements. polyphenols of green Standardized solid process is devoid water of of green. 99.9% versions are green available. green of are the polyphenols green found of the in tea extract, tea, cups standardized is 3min 90*C, 194*. is total gallate of (194*) (about 3mmHg each).

Cancer:

There is no conclusive of black tea (but not green tea) has been associated with a significant reduction in death from all cancers. There is limited evidence to suggest that green tea consumption maybe associated with a slightly lower risk of esophageal cancer in the Chinese population, lower risk of lung cancer in women, and lower risk of oral cancer in Asian people. In 2015 meta-analysis of nine prospective cohort studies concluded that a high amount of green tea consumption may be associated with a lower risk of liver cancer in Asian women.

Cardiovascular Disease:

Observational studies have shown a correlation between daily consumption of green tea and a lower risk of death from cardiovascular disease. In 2015 meta-analysis of such observational studies, an increase in one cup of green tea per day was correlated with a 5% lower risk of death from cardiovascular causes. Green tea consumption may be correlated with a reduced risk of stroke. Meta-analysis of randomized controlled trials have found that green tea consumption for 3-6 months appears to lower systolic and diastolic blood pressure a small amount (about 3mmHg each).

Glycemic Control:

Green tea consumption lowers fasting blood sugar but in clinical studies the beverage’s effects haemoglobin A1c and fasting insulin level was inconsistent.

Hyperlipidemia:

Drinking green tea or taking green tea supplements decreases the blood concentration of total cholesterol (about 7mg/dL), LDL cholesterol (about 2mg/dL) and doesn’t affect the concentration of HDL cholesterol. A controlled trials and concluded that green tea consumption lowers total and LDL cholesterol concentrations in the blood.

Mortality Risk:

Daily consumption of green tea is significantly correlated with a lower risk of death from any cause, an increase of one cup of green tea per day is linked with a 4% lower risk of death from any cause. A separate analysis found that an

Camellia sinensis

The colour of green tea brewed for 3min at 90*C (194*)

Regular green tea is 99.9% water, provides 1 calorie per 100mL serving, is devoid of significant nutrient content and contains phytochemicals, such as polyphenols and caffeine. Polyphenols found in green tea include epigallocatechin gallate (EGCG), epicatechin gallate, epicatechins and flavonols, known as kaempferol, quercetin and myricetin. A remarkable higher content of myricetin is detected in tea and its extracts than in many other plants, and its high concentration of myricetin may have some implications for the experimentally – observed bioactivity of tea and its extracts in vitro. Although numerous claims have been made for the health benefits of green tea, human clinical research has not provided conclusive evidence of any effects. In 2011 a panel of scientist published a report on the claim for health effects at the request of European Commission: in general they found that the claims made for green tea were not supported by sufficient scientific evidence.
increase of three cups of green tea per day was associated with a lower risk of total mortality in Asians and women.

Weight Loss:

There is no conclusive evidence that green tea aids in weight loss.

Toxicity:

Moderate, regular, and habitual consumption of green tea is safe, however there are reports of liver toxicity in humans after consuming high doses (10-20 mg/kg/day) of green tea extract dietary supplements, and high doses may act as pro-oxidant to damage DNA.

Production of Green Tea:

In 2013, global production of green tea was approximately 1.7M tonnes, with a forecast to double in volume by 2023. As of, China provided 80% of the world’s green tea market, leading to its green tea exports rising by 9% annually, while exporting 325, 000 tonnes in 2015. In 2015, US was the largest importer of Chinese green tea (6, 800 tonnes), an increase of 10% over 2014, and Britain imported 1, 900 tonnes, 15% more than in 2014. in 2015 Kenya was the largest exporter of green tea in the world (443, 000 tonnes).

Growing, Harvesting and Processing:

Green tea is processed and grown in a variety of ways, depending on the type of green tea desired. As a result of these methods, maximum amounts of polyphenols and volatile organic compounds are retained, affecting aroma and taste. The growing conditions can be broken down into two basic types- those grown in the sun and those grown under the shade. The green tea plants are grown in rows that are pruned shoots in a regular manner, and in general are harvested three times per year. The flush takes place in late April to early May. The second harvest usually takes place from June through July and the third picking takes place in late July to early August. Sometimes there will also be a fourth harvest. It is the first flush in the spring that brings the best quality leaves, with higher prices to match.

Green tea is processed using either artisanal or modern methods. Sun -drying, basket or charcoal firing, or pan-firing are common artisanal methods. Oven drying, tumbling, or steaming is common modern methods. Processed green teas, known as aracha, are stored under low humidity refrigeration in 30 or 60 kg paper bags at 0-5°C(32-41°F). This aracha has yet to be refined at this stage, with a final firing taking place before blending, selection and packaging take place. The leaves in this state will me refined throughout the years as they are needed, giving the green teas a longer shelf-life and better flavour. The first flush tea of May will readily store in this fashion until the next year’s harvest. After this re-drying process, each crude tea will be shifted and graded according to size. Finally, each lot will be blended according to the blending order by the tasters and packed for sale.

Foods are considered for their nutritive value. They show positive effects in protecting and preventing chronic diseases. Green tea and its health benefits play a role in oral cavity. High molecular weight polyphenols are isolated from green tea possess antioxidant, antibacterial cariostatic, antitumor activities.

In vitro shows green tea inhibits the growth and cellular adherence of cariogenic pathogens. Green tea intake helps in prevention and treatment of specific oral pathogens. Oral cavities consist 750 species of commensal micro-organisms mainly streptococcus mutans, streptococcus sobirinus. Health benefit of consuming green tea includes the prevention of cancer, antibacterial, antioxidative and lowering cholesterol. An antioxidant property of green tea prevents oxidative damage of DNA. The chemical composition of green tea consists of proteins, enzymes, amino acids, carbohydrates, minerals and trace elements (lipids, pigments, steroids, vitamins and volatile compounds). Fresh tea leaves contain alkaloids and catechins. The aim of this study was to assess the effects of green tea leaves extracts on the aerobic mouth bacterial load and to understand this we will take both green and black teas. Shows to inhibit the growth and activity of bacterial associated with mouth infection.

2. Methodology

Materials & Methods:

1. Leaves Drying Method and Preparation of Dried Extract Powder

We take a fresh green tea leaves and were spread out in the hot air to wither. Once they became soft and pliable, they were pan fried. In the final step, the leaves were dried by firing as far as the natural fragrances and flavours stabilized and leaves kept their green colour. After that the dried leaves were powdered by the grinder (mechanically) and then to obtain the extract, 200g of 18 mesh powders was added to 1000mL of deionized water at 70-80°C for 30min. After that extract were filtered, by a cloth filter, and concentrated under a low pressure condition installed in a laminar air flow and finally lyophilized (-40°C) by a freeze dryer for about 6 hours.
2. Tannin Assay, Validation & Preparation of Calibration Curve

Stock solutions of standard tannic acid were prepared. This process was repeated for 3-6 consecutive days to find the inter-day and intra-day variations; the absorption level was measured spectrophotometrically at 760nm.

3. Preparation of Equivalent Tannin from Green Tea Powder Extract

Sample containing 1% tannin was prepared by equivalent amount of 16.05mg of dried green tea powder, yielding from 100mg dried extract. The sample was poured into a 100mL stoppered bottle which has been sterilized by an autoclave at 121°C, 15lb pressure for 20min.

Volume of the sample was added to 100mL of deionized water & finally, filtered through a 0.45 micron membrane filter to be sterilized.

5. Preparation of Mouthwash Formulation

The quantity of 16.5mg green tea dried extract evaluate to 1% tannin was dissolved in about 70mL, 50°C deionized non-sugar sweetener.

The final volume as adjust to 100mL by deionized water after mixing thoroughly and prepare filtering. This formula was also repeated with the extra 10% propylene glycol.

The antimicrobial activity of PG formulation was compared with the chemical chlorhexidine 0.2% as the above method of extract.

6. Stability Evaluation

In order to examine the stability of green tea extract mouthwash containing 10% PG the mean of pH values, was recorded 48hours after formulation for 2weeks.

Also the recorded amount of tannin remained unchanged during 0-6 days holding at different temperatures (30°, 45° and 60°C)

4. Antimicrobial Evaluation of Green Tea 1% Extract

I had taken 2 girls students ageing 20-25 years asked to wash their mouth by 10-15mL deionized water for one minute at 12 'o' clock noon as a base time. After routine brushing their teeth with tooth paste at 10AM.

A mount content of each girl was poured into sterile conditions and than aliquots of 0.01mL of each sample was diluted in 1mL deionized water under sterile conditions and then sterile conditions and then an aliquot of each new sample was spread on petri – dishes containing blood agar. Plates were incubated inside of a candle jar with 5-7 CO2 concentration 35-37°C for 24 hours and then colonies were counted separately. This process was repeated for the extract containing 1% tannin exactly 2hours after washing the mouth with 10-15mL deionized water.

Collecting Sample of saliva

Autoclave Machine
3. Results

![Graph of bacterial load reduction](image1)

Mean value of bacterial load

![Graph of decreased bacterial load](image2)

Mean of decreased bacterial load

Figure 1

1. 143% (Water zero time)
2. 140% (Water 1 hour time)
3. 163% (Water 2 hour time)
4. 85% (Green tea extract 1%)

Figure 2

1. Tannin 1% without propylene glycol
2. Tannin 1% with propylene glycol
3. Chlorhexidine 0.2%

The result of mean for the assay of tanning in green tea extract repeated three consecutive days tannin in 100mg of dried extract powder of green tea.

The effectiveness of water as a negative control or drug free mouthwashes in different times of mouth washing (1 hour & 2 hour) after beginning the trial of zero time Figure 1.

The comparative result between water as a negative or drug free control and green tea 1% tannin concentration as the most effective antibacterial agent in green tea leaves shown in Figure 1.

The comparative results of the mouthwashes containing 1% tannin showed about 64% of bacterial reduction for the mouthwash containing 10% PG and 48% for PG free mouth wash. Evaluation of the effectiveness of the green tea mouthwash 1% tannin containing PG in comparison with the chemical chlorhexidine 0.2% mouthwash showed a higher and significant reduction on the bacteria Figure 2.

The result of stability evaluation of the product indicate that the amount of tannin remained unchanged during 14 days holding at different temperatures.

The pH evaluation of green tea extract mouthwash showed a constant value.

The mean value of tannin 1% remaining unchanged during 14 days, at different temperatures (30°, 45° and 60°C).

4. Discussion

1. Green tea contains flavonoids, tannin, vitamins, fluoride and other mineral salts. Some of antioxidant and antimicrobial agents of green tea could increase the life and efficiency of teeth. Tannins are biosynthetic materials which have a potent anti-bacterial effect.
2. In the recent tooth decay researches, it was mentioned that green and black tea may prevent formation of bacteria in mouth and therefore may reduce construction of plaques on teeth.
3. Also, it was emphasized that the routine consumption of green tea in humans under study might have reduced the intensity of teeth caries.
4. In our study, which specified the role of propylene glycol as a co-solvent instead of the ethyl alcohol, the result of green tea mouthwash showed a good reduction in bacterial colonies.
5. For example, researches on green tea in the United States of America, using green tea containing 0.5% tannin with 2% alcohol decreased the different types of *staphylococcus* just about 15%.
6. PG free tannin 1% green tea mouthwash we had about 48% and 64% reduction in the bacteria when 10% PG was used.
7. Green tea may contain higher amount of tannin or could be better formulated, quantitatively.
8. Using PG instead of harmful ethyl alcohol is not only useful as co-solvent but also is powerful chemical stabilizer, as well as intensifying the microbial death with safety for children and pregnant women.
9. Applying the simple 1% tannin increase the death of microbial death around twofold and even be extended to threefold when 10% propylene glycol is in charge.
10. Propylene glycol could improve the physic-chemical stability and shelf life of the product.
11. In this study, a comparison was done between green tea extract and water as a negative control to make sure that water had not influenced the death or removal of the bacteria during rinsing out the mouth with water.
12. This evaluation also was to find the best time for starting the trial with green tea extract.
1. The results of rinsing mouth just with water at different times (e.g. 0.1 and 2 hours) were analysed to find out the time interval of coming back the bacteria to about zero time, again. Therefore, the time interval for the bacteria to come back to the same amount of starting time was obtained about 2 hours.

2. So, the reason of choosing this lag time between the trials or washing the mouth with water 2 hours before testing with mouthwashes was as mentioned.

3. The comparison of the mouthwash containing 1% tannin with regular water and tannin extract alone.

4. The mean reduction of bacterial load mouthwashes containing 1% tannin with and without propylene glycol demonstrated the significant difference.

5. The effect of PG and its influence on activity of the extract on bacteria or its role as a co-solvent in the extraction, are quite clear.

6. The comparison of chlorhexidine 0.2% with water and tannin 1% containing PG with chlorhexidine 0.2% showed more significant difference on the mean reduction of bacterial load.

7. This great difference was because of using 10% polyethylene glycol in the mouthwash and of course it can help the strength.

8. It should be noted that the difference between the PG free 1% tannin of green tea extract and chlorhexidine 0.2% was not significant.

9. Incorporation of the propylene glycol in a water solution as a co-solvent could increase the efficiency of extraction process, especially in the case of oily material of plants (essential oil), in addition to increasing the shelf life and stability of the product.

10. To make sure the stability of the mouthwash is constant after formulation, the evaluation of green tea mouthwash pH must not show significant change at different times.

11. The study of chemical stability for green tea mouthwash was followed by calculating the amount of tannin remained unchanged, during zero to 90 days after holding the mouthwash at 30, 30, 45 and 600 C temperature in separate testing conditions.

1. Generally, any dosage from which could have drug activity as much as 90% of the original dose of the formulation, said to be and legally authorized by official use.

2. Studies showed that green tea, due to its ability to remove the mouth microbial contamination, can eliminate bad breath or halitosis.

3. Green tea helps toothpaste and mouthwashes fight viruses by eliminating bacteria.

4. It also helps to prevent plaque formation within gums and teeth.

5. Plaque is another contributor of bad breath.

6. Green tea may prevent bad breath by daily consumption, using it as a mouthwash before and after brushing teeth, or mixing it with the toothpaste products.

7. A herbal mouthwash formulation of green tea extract containing 1% tannin with 10% propylene glycol could reduce the aerobically mouth bacterial load as much as 45-64% and also, due to this reduction it may prevent plaque formation on teeth and consequently, halitosis.

8. This last claims need to be approved by further study.

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10. Replacement and incorporation of propylene glycol in this study for ethyl alcohol is not less harmful, especially for children and pregnant women, but it can also influence or increase the strength and antimicrobial effect of green tea extract as well as its stability.

5. Conclusion

Using green tea extract as a herbal mouthwash is safe and harmless specially for children and pregnant women. This result led us to suppose that green tea may prevent plaque formation on teeth, coming over halitosis due to mouth infection.

Mouth wash widely recommended by dentists as an essential part of your oral health routine but recent studies hint it may be doing more harm than good.

Studies shows that the regular use of alcohol-based mouthwash may lead to mouth cancer, which can affect the tongue, the floor of the mouth, the gums and the cheeks.

More than 640, 000 people across the world are diagnosed with the disease every year.

What is mouthwash?

While each company has their own unique mixture of ingredients, here are a few that often appear together on the label of popular brands like Listene and Colgate.

1. Chlorhexidine

According to a study published in the Journal of International Academy of Periodontology, minimal exposure to his chemical can cause headache, euphoria, giddiness, blurred vision, stomachache, and complete loss of taste for a minimum of 8 hours after ingestion.

A Free Radical Biology and Medicine Journal confirm that the chemical also kills off the good bacterium that helps blood vessels relax and maintain blood pressure. Hence, chlorhexidine raises blood pressure and makes you more likely to suffer from a heart attack, heart failure, or stroke.

Using a mouthwash twice daily can increase blood pressure by up to 3.5 mmHg, to put it in perspective, a two – point rise in blood pressure can elevate the risk of dying from stroke by 10%, and of heart disease by 7%.

Not all mouthwashes contain the chemical, but the study authors caution other mouthwashes could still produce the same effects as Corsodyl by damaging the mouth’s healthy bacteria.
2. Alcohol

Alcohol stresses out the liver and dries out the mucous membranes in your mouth, it also reduces saliva production and changes the pH of the mouth and throat, causing bad breath.

The Dental Journal of Australia concluded in a review that ‘that alcohol-containing mouthwash contributes to the increased risk of development of oral cancer’.

And that ‘that it is inadvisable for oral healthcare professional to recommend the long-term use of alcohol-containing mouthwashes’.

3. Hexetidine (oraldene)

This chemical causes skin, eye and respiratory irritations while also being cytotoxic. When present oral rinses, Hexetidine that interferes with cell division and vital metabolic processes.

It’s also known to cause blood clots I the brain, unstable heartbeat, and allergic reactions.

4. Methyl Salicylate

Methyl salicylate can cause an allergic reaction with symptoms like rash, hives, itching, shortness of breath, wheezing, cough, swelling of the face, lips, tongue, or throat or any other signs.

It should never come into contact with open wounds like cheek bite and flossing injuries.

It takes very little quantities of the chemical to overdose and the consequences can be deadly.

Side effects include tinnitus, nausea, and vomiting, coma, convulsions, hyperventilation, and more severely, pulmonary edema, haemorrhage, acute renal failure or death.

Swallowing mouthwash containing Methyl Salicylate can also cause gastric ulcers, gastrointestinal haemorrhage and erosive gastritis.

5. Methylparaben

Methylparaben is often found in mouthwash, cosmetics and deodorants.

It has potentially carcinogenic properties as it’s been found in breast cancer tissue and was discovered to increase chromosomal aberrations in ovary cells during lab testing.

It also reported to produce an estrogenic response and affect sperm quality.

The Natural Solution

A big part of oral hygiene isn’t just about brushing your teeth and getting enough calcium and vitamin D. It also has a lot to do with what you eat.

Studies have shown that eating a diet rich in veggies, fruits and whole grains effectively reversed gum inflammation and reduced instances of periodontal disease.

As they say, prevention is best medicine!

If you have sensitive gums and you need a little extra help, take out your kettle! Researchers have found that rinsing your mouth with green tea inhibited the growth of plaque bacteria in less than 10 minutes. This is because green tea has antibacterial and anticariogenic properties that decreases the acidity of your saliva and kills plaque. Tea even works better than chlorhexidine in reducing plaque and is much safer to use.

How to Make Green Tea Mouthwash (Home Made)

Ingredients

- 1.5 L of water
- 7 tablespoons of green tea
- A few tablespoon of dried peppermint (optional)
- A strainer
- A 2L glass bottle
- A large bowl
Instructions

- Boil the water in a large saucepan.
- Measure out the tea and place it directly in the water. If you want your mouthwash to have a minty taste, add the peppermint leaves at the same time. For a strong mint flavour, mix equal part tea and mint.
- Let the mixture steep for one hour.
- As the tea steeps, wash out your bottle with hot water and vinegar and rinse well.
- Place the strainer above your bowl and pour the tea through it.
- Add ½ L of water and pour into bottle.
- Store in the refrigerator.
- To use, measure out an ounce (30mL) of the mouthwash, swish around for ten minutes and spit it out.

6. Summary

Different studies used green tea extract.

Study 1:

How green tea helps keep a high oral pH, lowers (cavity causing) S. mutans population and reduce oral bleeding.

Study 2:

Another study showed how green tea dramatically reduces plaque accumulation and improves gingival index and improves salivary pH.

Study 3:

In the former study, oral pH removed above 6 (mildly acidic) without green tea, but plummeted below 5 without (relatively very acidic).

Study 4:

In the later study, mean plaque score goes from 1.45 to a ridiculously low 0.11, indicating a 94% reduction in plaque.

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