

**PREPARATION OF MOUTHWASH USING *CAMELLIA SINENSIS* (GREEN TEA)
AND *CARICA PAPAYA* (PAPAYA) HERBAL FORMULATION AND ITS
ANTIMICROBIAL ACTIVITY**

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Abstract :

Aim: To compare antimicrobial potential of *Camellia sinensis* (Green Tea) and *Carica papaya* (papaya) herbal formulated mouthwash.

Background: Dental caries and periodontitis is considered to be common and highly persistent human diseases confined to the oral cavity. Adequate reduction in the prevalence of the pathogens responsible for dental caries and periodontal disease is required which can be achieved through effective strategies such as interdental cleaning, brushing teeth regularly and using commercial mouthwashes with antimicrobial properties. Green tea and papaya has been accounted for to be helpful for avoidance of periodontal disease and to prevent dental caries. Green tea might be beneficial for treating mouth ulcers since it has antiinflammatory properties. It may be useful to clean the mouth, relieve pain, and stop the bleeding of the gums.

Materials and Methods: The plant extract was prepared with 1g dried green tea leaves and 1g powder of papaya leaves mixed with 100ml distilled water and boiled for 10 minutes, at 60-80°C on a heating mantle. Filtration of the extract was done using Whatman no.1 filter paper. Further the herbal formulation of Green tea and papaya and commercial mouthwash was subjected to determine the antimicrobial activity.

Results: High zone of inhibition of mouthwash prepared from green tea extract and papaya was observed. It mainly showed increased effectiveness against *Enterococcus faecalis* and *Candida albicans* whereas commercial mouthwash showed

Conclusions: Increased Green tea and papaya herbal formulated mouthwash has good antimicrobial activity against streptococcus mutans, staphylococcus aureus, candida albicans and enterococcus faecalis when compared to commercial mouthwash.

Keywords : Antimicrobial activity, papaya, Herbal formulation, Green tea.

Introduction:

The oral cavity provides an environment for the accumulation and proliferation of the microorganisms which later tends the pioneer for causing dental caries when favorable environmental changes occur¹. It is also responsible for the formation of biofilm around the teeth surface and gingival tissues. Lifestyles, types of food intake, frequency of food intake, personal and oral hygiene, gastrointestinal diseases play an important role in the development of plaque and dental caries which remains very common in the Indian population². The prevalence of the oral pathogens responsible for causation of plaque formation can be successfully reduced through effective strategies such as cleaning, using interdental aids, maintenance of proper oral hygiene and using mouthwashes with high antimicrobial properties³. Continuous use of the antimicrobial property containing mouthwash showed improved resistance over several bacteria in the oral cavity⁴. The commercial mouth contained antibacterial agents such as essential oils such as oregano, thyme, lemon and lavender, and Cetylpyridinium chloride [CPD]^{5,6}.

Periodontitis refers to an infectious condition which is diagnosed with marked inflammatory response of the gingival and periodontal tissues leading to the destruction of the tissues^{7,8}. The main objective of the periodontal therapy is to destroy the pathogens which are responsible for the disease and to maintain proper periodontal health⁹. The maintenance of periodontal health can be achieved by either nonsurgical or surgical therapy. Antibiotic therapy stays as the first line of defense used to control oral pathogens responsible for periodontitis¹⁰. Antibiotic therapy has been used in the late 1970s for treating periodontal problems and a high success rate was observed¹¹. Therefore, antibacterial properties of the mouthwash are necessary for the bacterial specificity in certain types of periodontitis which determines rate of progression of the disease. Mouthwashes with antimicrobial properties are thus used as an adjunct therapy for prevention and maintenance of periodontal health problems¹²⁻¹⁴. The mouthwash with antimicrobial activity mainly aims at destruction and suppression of the bacterial proliferation.

Many herbal extracts are used as mouthwash such as German chamomile, triphala, peppermint satva, Terminalia chebula, Green tea, guava extract, Aloe vera, turmeric, neem, pomegranate extracts, propolis, and alum. These herbal extracts are proven to be used as single or in various combinations as herbal mouth wash. It is mainly preferred because of the safety and effectiveness against oral health problems such as bleeding gums, mouth ulcers, halitosis, and preventing dental caries. It has also proven that the side effects are very low compared to chemical mouthwashes. *Carica papaya* (commonly known as papaya) are herbaceous plants belonging to the members of the *Caricaceae* family. It is a fast growing herb with a short life span with all parts of the plant such

as fruit, seed leaves, root, stem, and flower being significantly used for the treatment of different types of diseases^{15,16}. The *C.papaya* flowers have useful medicinal properties and can prevent cancer, increase digestion and appetite, and delineate heart problems. The tannins, flavonoids, and antioxidants in papaya flowers have been described previously to knock out free radicals from the body. Consumption of papaya flowers helps the body to neutralize the free radicals and modulate the immune system increasing disease susceptibility¹⁷. *Carica papaya* contain phytochemicals which have high anti-inflammatory, antibacterial and antiviral properties. Most of the herbal formulated mouthwashes contain phytochemicals which help in the reduction of oral pathogens¹⁸⁻²⁰.

Green tea (*Camellia sinensis*) is popularly used worldwide for the antiinflammatory property. It also has anticancer properties. Among the active compounds present in green tea, catechins are the major antioxidants²¹. Recent studies on the biological compound in green tea revealed that the presence of a number of hydroxyl groups have a major impact on the antimicrobial property and antioxidant property. Unfermented green tea is mostly preferred in the preparation of mouthwash due to its bioavailability of catechins and other anticariogenic agents²². Catechins show neutralizing properties of reactive oxygen and nitrogen species. The group of green tea catechin derivatives include, epigallocatechin, epicatechin gallate and epicatechin²².

The antimicrobial activity of green tea was also enhanced by the membrane perturbation. Increased dose of antibacterial ECGG damages the liposome membrane of *E.coli* and *S.aureus*, which results in the leakage of the intramembranous materials and aggregation of the liposomes in the cellular matrix²³. Studies also show that total protein present in green tea inhibits *Pseudomonas aeruginosa* through damage to the cell membrane with the release of small cellular molecules. Epigallo galactin (EGCG) and Epicatechin gallate (ECG) strongly inhibit biofilm formation of commensal and pathogenic *E. coli* colonies, thereby reducing the expression of CsgD - a crucial activator of curli and cellulose biosynthesis²⁴. The main aim of the study is to observe the antimicrobial activity of combined effects of papaya and green tea formulated mouthwash.

Materials and method:

Herbal formulation preparation:

preparation of extract was done by 2 g of papaya and green tea diluted in 100 ml distal water. it boiled for 10 minutes at 75 degree celsius. the extract is then filtered and concentrated.

Mouthwash preparation:

preparation of mouthwash was done by adding 0.3 g sucrose 0.01 g sodium lauryl sulfate agent (foaming agent) + sodium benzoate in 10 ml of extract prepared.

Antimicrobial activity:

streptococcus mutans, streptococcus faecalis, streptococcus aureus and candida albicans are the bacteria and fungi used in this activity. organisms will be cultured using standard methodology. minimum inhibitory concentration (mic) and minimum bactericidal concentration (mbc) will be studied using a well diffusion method.

Antibacterial activity of mouthwash:

The agar well diffusion method was used in antibacterial activity of papaya and green tea mouthwash and commercial mouthwash. different concentrations of papaya and green tea mouthwash and commercial mouthwash were tested against streptococcus mutans(gram +), streptococcus faecalis, and streptococcus aureus. the fresh bacterial suspension was dispersed on the surface of muller hinton agar plates. different concentrations of prepared mouthwash (25,50&100µl) were incorporated into the wells and the plates were incubated at 37°C for 24 h. the antibiotics were used as positive control. zone of inhibition was recorded in each plate.

Antifungal activity of mouthwash:

The candida albicans fungal was isolated using dilution method. the agar well diffusion method and disc diffusion methods were used to determine the antifungal activity of papaya and green tea mouthwash and commercial mouthwash. different concentrations of papaya and green tea mouthwash and commercial mouthwash was tested against the oral pathogenic candida albicans. the fresh fungal suspension was dispersed on the surface of rose bengal agar plates. different concentrations of prepared mouthwash (25,50 &100µl) were incorporated into the wells and the plates and discs were incubated at 37°C for 48 h. Antibiotics were used as positive control. zone of inhibition was recorded in each plate.

Results and discussion:

The zone of inhibition is a circular area around the spot of the antibiotic in which the bacteria colonies do not grow. The zone of the inhibition of the green tea and papaya formulated mouthwash was tested against streptococcus aureus, staphylococcus mutans, Enterococcus faecalis and Candida albicans. The zone of the inhibition was compared with concentrations 25µL, 50µL, 100µL against the control group. The control group refers to the commercial mouthwash which was available in the market. The increase or decreases in the zone of inhibition of the prepared mouthwash demonstrates the effectiveness of the mouthwash which has been used to increase the resistance and control of microorganisms in the oral cavity.

The following figures shows the zone of inhibition of the formulated mouthwash with various microorganisms



Figure 1- Antimicrobial property against *S. aureus*.

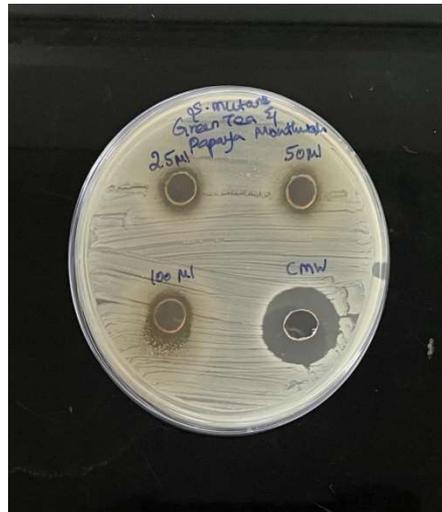


Figure 2- Antimicrobial property against *S. mutans*

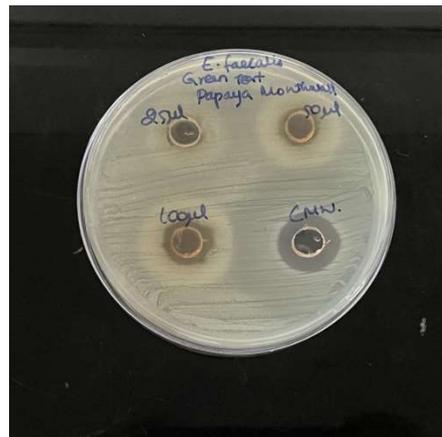


Figure- 3- Antimicrobial property against *E. faecalis*



Figure 4- Antimicrobial property against C.albicans

	25	50	100	CMW
C. Albicans	18	20	24	15
S. aureus	9	10	13	20
S. mutans	13	13	14	21
E. faecalis	9	12	15	18

Table-1 Zone of inhibition and Antimicrobial activity of green tea extract and papaya herbal formulated mouthwash

From the above results, considering the zone of inhibition against *S. mutans*, the zone of inhibition against 25µL of solution is 13 mm, zone of inhibition against 50µL of solution is 13mm and zone of inhibition against 100µL is 14 mm whereas the zone of inhibition of control is 21 mm. The zone of inhibition against *S.aureus* is 9 mm for 25µL of solution, 10mm for 50µL of solution and 13mm for 100µL of solution whereas 20mm for control group. Considering the zone of inhibition against *E.faecalis*, the zone of inhibition against 25µL of solution is 9 mm, zone of inhibition against 50µL of solution is 12mm and zone of inhibition against 100µL is 15 mm whereas the zone of inhibition of control is 18 mm. The zone of inhibition against *C.albicans* is 18 mm for 25µL of solution, 20mm for 50µL of solution and 24mm for 100µL of solution whereas 15 mm for control group. Comparing the zone of inhibition against various microorganisms, it is observed that the zone of inhibition increases with the concentration of mouthwash. Comparing the microorganisms, *C.albicans* showed greater zones of inhibition which indicated enhanced antifungal properties of the mouthwash can be observed.

Zone of Inhibition testing is a fast, qualitative means to measure the ability of an antimicrobial agent to inhibit the growth of microorganisms. In the world of antimicrobial substances/surfaces, the degree to which these materials are inhibitory can be of vital importance to the health of the consumer²⁵. This test is an outstanding qualitative way for manufacturers of antimicrobial surfaces/substances to be able to compare the inhibition levels of their products. Few studies have demonstrated that green tea plays an important role in the counteraction of the damage to dental tissues such as gingival tissues, alveolar bone and periodontal ligaments²⁶. The counteraction of the green tea is mainly against the pathogenesis of periodontal disease. Evidence of decrease in gingival bleeding, decrease in the depth of the pocket has been noticed with the help of green tea and papaya mouthwash. It also helps in advancing the periodontal healing during periodontal therapy²⁷. The zone of inhibition of the green mouth was 8 times greater than the commercial mouthwash which has been tested against streptococcus mutans whereas in our study it is evident that the effectiveness of the mouth wash is greater against candida albicans compared to other microorganisms.

Several herbal mouthwashes are used nowadays as a single component or in combinations. The combination of *Camellia sinensis* and *Carica papaya* based herbal mouthwash has not been explored. Both green tea and papaya has its antimicrobial and antioxidant properties which makes it suitable to use as combinational mouthwash. The additive effect helps in prevention from development of dental caries and helps in healing phase during periodontal therapy. The combination of papaya and green tea mouthwash can be used as an assistant for the periodontal therapy in recurrent periodontitis and even during oral prophylaxis²⁸. Lack of proof of toxic impacts for example, burning sensation, irritation of oral mucosa or gingival tissues, vesicles was noticed. The utilization of the mouth prepared was very much secure, easy to use and affordable²⁷. Comparing herbal and chemical mouthwashes, 90% of the chemical mouthwashes contain alcohol and fluoride which is considered to have toxic effects on the body in overdose. Hence, most herbal mouthwashes are a safe alternative to pregnant women, people with dry mouth, diabetic and to children.

By comparing the antimicrobial activity of green and papaya herbal formulation-based mouthwash and commercial mouthwash, it can be concluded that green tea and papaya herbal formulated mouthwash shows good antimicrobial activity when compared to commercial mouthwash. As the concentration of extract increases, the zone of inhibition also increases.

Conclusion:

Antimicrobial activity of green tea extract and papaya formulated mouthwash has good Antibacterial activity as there is a high zone of inhibition in *Enterococcus faecalis* and *Streptococcus aureus*.

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