

COMPARATIVE EVALUATION OF ANTI-MICROBIAL EFFICACY OF GREEN TEA EXTRACT AND CHLORHEXIDINE MOUTHWASH ON RED COMPLEX, ORANGE COMPLEX AND GREEN COMPLEX PERIODONTAL PATHOGENS – AN IN-VITRO STUDY

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ABSTRACT:

Introduction: Green tea is a popular beverage nowadays and intake of green tea polyphenols has shown preventive effect against cancer and cardiovascular disease in experimental and epidemiologic studies. Many people have been switching their morning routines from coffee to green tea to take advantage of its multiple health benefits.

Aim And Objectives: To comparatively evaluate the antimicrobial efficacy of green tea extract with Chlorhex (0.2% chlorhexidine mouthwash) on gram negative periodontal pathogens of *P.gingivali*, *P.Intermedia* and *Agrragtibactor Actinomycetemcomitans*.

Materials and method: A total of 18 samples (6 groups with 3 pathogenic strains) were included in the study. There were 3 groups of organisms, tested on 6 different reagents: 5mg/ml Green tea extract, 10mg/ml Green tea extract, 15mg/ml Green tea extract, 20mg/ml Green tea extract, 0.2% Chlorhexidine Mouthwash and Distilled water. Each sample was repeated 3 times to obtain a mean value. The diameter of zones of inhibition was measured for all the wells using a Digital Vernier Calipers. The mean score of zones of inhibition were calculated for each solution respectively.

Results: 0.2% Chlorhexidine mouthwash showed greatest zone of inhibition against all periodontal pathogens. Green tea at a concentration of 5mg showed antibacterial activity against *P. Gingivalis*, *P. Intermedia* and *A. Actinomycetemcomitans* while at a concentration of 10mg and 15mg antibacterial activity was seen against *P. Gingivalis*, and *P. Intermedia*. *A. Actinomycetemcomitans* was resistant to 10mg, 15mg and 20mg concentration.

Conclusion: Based on the results obtained from the present research it can be terminated that green tea extract, does indeed possess antimicrobial activity against the periodontal pathogens *P.gingivalis*, *P.intermedia* and *A.actinomycetemcomitans*.

Keywords: Green Tea, Red Complex, Green Complex, Orange Complex, Chlorhexidin

INTRODUCTION:

The delightful taste and anticipated health effects has made tea the most famous drinks this days. Going back to 4,000 years ago in Asian cultures, where green tea was acclimated to heal wound and cure diseases by many age-old Japanese and Chinese health practitioners'. It has been apparent well accepted by many recent studies that enormous benefits ranging from weight loss to cancer prevention can be overcome by a cup of green tea. Many humans have been shifting their morning routines from coffee to green tea to yield advantage of its numerous health benefits.

Bags of bioactive ingredients present in green tea, mainly polyphenols, play a key role in the avoidance and cure of many diseases. Catechin that belongs to a group of polyphenol is the active compound in green tea. Epicatechin gallate (ECg), epigallocatechin gallate (EGCG), epigallocatechin and epicatechin are the four catechins present in green tea. Green tea is important because of its antioxidant potential and mechanisms by which it operates as antioxidant. Carotenoids, ascorbic acid, tocopherols, and minerals like magnesium, chromium, zinc, and selenium are also present in green tea. It

also incorporates caffeine. In a cup of green tea the bulk of caffeine will vary depending on the bulk of tea used, the length of time the leaves are infused and if a person drinks the first or second infusion. Green tea acts by constraining the activity of the reactive oxygen species particle thus stops all the degenerative changes.

Periodontal health has been associated with Gram positive coccoid bacteria, while Gram negative rods and spirochetes have been related to periodontal disease. Evidence have shown that various bacteria including *A. actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Campylobacter rectus*, *Peptostreptococcus micros*, *Fusobacterium nucleatum*, *Eubacterium nodatum* and various spirochetes, such as *Treponema denticola*, have their role in commencement and advancement of periodontal disease. Key periopathogens *A. actinomycetemcomitans* (green complex), *Tannerella forsythia* (red complex), *Prevotella intermedia* (orange complex) and *Porphyromonas gingivalis* (red complex), are actively linked with the

commencement of periodontal disease, and disease advancement⁶.

Considering the treatment of periodontal disease that is scaling and root planning and mechanical plaque control some patients do not respond well to the treatment. At times antimicrobial substances or products have been recommended as an adjuvant to periodontal therapy. Numerous oral active antimicrobial substances have been discovered and used to avoid and diminish the construction of dental plaque. Numerous researches have proved that Chlorhexidine gluconate is the most effective agent and is still examined intensely. Duration of its use is limited due to its undesirable effects like taste disturbances, tooth discoloration and mucosal erosions, but the presence of the active ingredient; and because of its outstanding substantivity, chlorhexidine is considered to be the utmost efficient agent for diminishing plaque and gingivitis. 0.2% chlorhexidine gluconate solution is thus considered to be the gold standard and is mostly used in researches as a positive control⁷.

To overcome several limitations of chlorhexidine special attention is been inclined to the use of natural medication. Green tea has been widely studied and

been used for therapeutic purpose in various branches of medical sciences, as well as in dental sciences for dental caries and gum disease. Researchers believed that the antioxidant Catechin reduces the severity of periodontal (gum) disease. Green tea naturally protects the teeth and gums by inhibiting the growth of oral bacteria and plaque.

Among the various natural products, green tea has received greater attention due to its broad-spectrum antimicrobial activity against wide range of pathogenic microorganism. It is suggested that green tea extract could be same as a new source of natural antimicrobial and antioxidant with potential applications for reducing the levels of periopathogens.

Insufficient evidences are available to support antimicrobial property of different concentrations of green tea extract in context to red, orange and green complexes of periodontal pathogens and even comparison of same with clinically used and accepted 0.2% chlorhexidine. This in-vitro research was planned to figure out antibacterial efficacy of green tea extract in 5, 10, 15, 20 mg/ ml and to compare that with 0.2 % (2 mg/ml) chlorhexidine (Chlorhex) on Red complex(*P. gingivalis*) , Orange

complex(*P. intermedia*) and Green Complex (*A. a*) periodontal pathogens.

Materials and method:

The study was started after Institutional Ethics Committee approval was obtained. An in-vitro study was carried out in the Department of Molecular Biology and Immunology, Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences And Research Centre, Belgaum, Karnataka. Written permission was obtained from Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences And Research Centre, Belgaum, Karnataka for 'extract preparation' and 'microbiological analysis'. A total of 18 (6 groups with 3 pathogenic strains) were included in the study. There were 3 groups of organisms, tested on 6 different reagents: 5mg/ml Green tea extract, 10mg/ml Green tea extract, 15mg/ml Green tea extract, 20mg/ml Green tea extract, 0.2% Chlorhexidine Mouthwash and Distilled water. Each sample was repeated 3 times to obtain a mean value.

Preparation of Extract:

Green tea extract was prepared in College of Pharmacy, Sumandeep Vidyapeeth, Gujarat. To finely ground powder of dried green tea leaves (100 gm) double distilled hot water (60°C) was

added in the ratio of 1:5 with occasional stirring. The boiling mixture's filtrate was collected (two times). The filtrate was concentrated till dryness. The dried powder was kept in a desiccator.

250 mg of green tea aqueous extract was aseptically weighed and dissolve in 10 mL of 10% DMSO solution. The stock solution (25mg/ml) was serially diluted with 10% DMSO to attain different concentrations of 5, 10, 15 and 20 mg/ ml respectively. The contents of the tubes was properly mix by using vortex mixer.

Disc Diffusion Method:

The antibacterial activity of green tea extract and 0.2% chlorhexidine was determined by disc diffusion method. Culture medium used was Blood agar. A loop or swab method was used to transfer microbial colonies to the agar plates. Turbidity was visually adjusted with the broth to equal that of a 0.5 McFarland turbidity standard that had been vortexed.¹ Alternatively, the suspension was standardized with a photometric device. Within 15 minutes of adjusting the inoculums to a McFarland 0.5 turbidity standard, a sterile cotton swab was soaked into the inoculum and rotated against the tube wall above the liquid to remove excess inoculum. Then swabbing the entire surface of the agar plate was done thrice,

rotating plates approximately 60° to ensure even distribution. Care was taken to avoid extra hitting of the sides of the plates to prevent creating aerosols.

Inoculated plates were allowed to stand for at least 3 minutes but no longer than 15 min before making wells. A hollow tube of 5mm diameter was heated and pressed above the inoculated agar plates and was removed immediately, making a well in the plate. Likewise, four wells of 5mm diameters each were made on each plate. Add 50 µl of compound (5mg, 10mg, 15mg and 20mg), Distilled water and Chlorhexidine into the respective wells on each plate. Within 15 min of compound application, plates were shifted to an anaerobic jar, which was kept in an incubator at 37⁰C for 48 hours. After incubation was complete, plates were read only if the lawn of growth was confluent or nearly confluent. The diameter of zones of inhibition was measured for all the wells using a Digital Vernier Calipers. The mean score of zones of inhibition were calculated for each solution respectively.

Observations and results:

The mean value of diameters of zone of inhibition of 5 mg/ml green tea

extract, 10 mg/ml green tea extract, 15 mg/ml green tea extract, 20 mg/ml green tea extract, 0.2% Chlorhexidine mouthwash and Distilled water are shown in table 1.

0.2% Chlorhexidine mouthwash showed greatest zone of inhibition against all periodontal pathogens. Green tea at a concentration of 5mg showed antibacterial activity against *P. Gingivalis*, *P. Intermedia* and *A. Actinomycetemcomitans* while at a concentration of 10mg and 15mg antibacterial activity was seen against *P. Gingivalis*, and *P. Intermedia*. *A. Actinomycetemcomitans* was resistant to 10mg, 15mg and 20mg concentration.

Figure 4: Zones of inhibition of Pg at different concentrations of green tea extracts on periodontal pathogens

Figure 5: Zones of inhibition of Pi at different concentrations of green tea extracts on periodontal pathogens

Figure 6: Zones of inhibition of Aa at different concentrations of green tea extracts on periodontal pathogens

Figure 7: Zones of inhibition of Pg, Pi and Aa at different concentrations of green tea extracts on periodontal pathogens

Table 1. Mean values of diameter of zone of inhibition (R* = resistant)

		ZONE OF INHIBITION					
S	Name	5	10	15	20	D	C
l.	of the					/	H
N	microor	m	m	m	m	W	X
o	ganism	g	g	g	g		
.	s						
1	Pg	12 m m	10 m m	8 m m	R	R	18 m m
2	Pi	8 m m	10 m m	10 m m	R	R	18 m m
3	Aa	8 m m	R	R	R	R	18 m m

Figure 1: Zones of inhibition of Pg at different concentrations of green tea extracts on periodontal pathogens

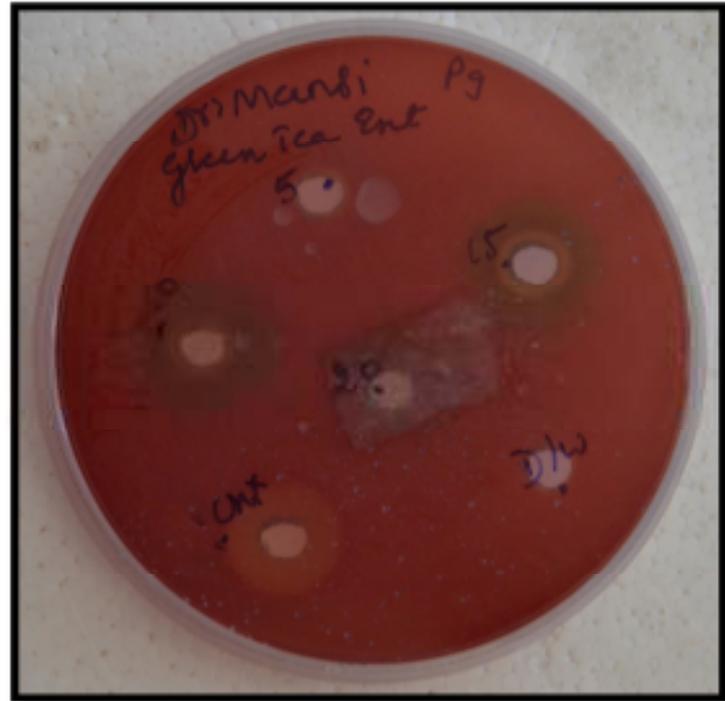


Figure 2: Zones of inhibition of Pi at different concentrations of green tea extracts on periodontal pathogens



Figure 3: Zones of inhibition of Aa at different concentrations of green tea extracts on periodontal pathogens



Figure 4: Zones of inhibition of Pg, Pi and Aa at different concentrations of green tea extracts on periodontal pathogens



DISCUSSION:

It was quoted by Rabbani et al, (1981) that left over bacterial plaque and calculus after scaling leads to the failure of periodontal treatment.³ Therefore to treat periodontal diseases the use of drugs is advocated^{7, 8}. Available anti-plaque agents are based on the use of broad-spectrum antimicrobial agents like chlorhexidine, quaternary ammonium compounds, antibiotics, etc. These synthetic antimicrobial agents have resulted in considerable side effects, antimicrobial resistance and the emergence of previously uncommon infections owing to their improper usage. Various studies on green tea in the past have shown the beneficial effects of green tea as a health drink. Green tea extracts have been exhibited to hold anti-inflammatory, anti-carcinogenic, antioxidant, and anti-microbial properties¹⁰.

The results of the present research showed that green tea extract have potent antibacterial activity on Pg, Pi and Aa, the causative bacterias for periodontal disease. Polyphenols present in green tea extract has antiplaque activity. The polyphenols includes the catechins, which are anticipated to be answerable for the health benefits that have generally been attributed to green tea. The most active and abundant

catechin in green tea is epigallocatechin-3-gallate (EGCG).⁴ Microbial plaque present on the teeth plays a very crucial part in the pathogenesis of periodontal disease. Some of the in vitro researches have shown that green catechin inhibits the production of *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Prevotella nigrescens*, as well as affects the adherence of *P. gingivalis* onto the human buccal epithelial cells⁶. The polyphenols present in green tea have shown to inhibit the production of toxic metabolites of *P. gingivalis*.⁵

Results of the present in vitro study showed that green tea extract inhibits Pg, Pi, and Aa activity at different concentrations. Catechins present in green tea plays a very important role in inhibiting these bacterial from causing periodontal destruction. These bacterias have been firmly involved in ruining of periodontal tissues and their cutback can lead to advancement in periodontal health status⁹.

Consumption of green tea or the use of green tea mouthwash has shown effective antibacterial effects when compared to chlorhexidine gluconate rinse. It was suggested that there was no evidence of irritation or burn reported with the use of green tea mouthwash and was

safer and economical compared to chlorhexidine gluconate rinse.

Green tea can abolish the production of osteoclasts by prohibiting the discharge of matrix metalloproteinases (MMPs) by osteoblasts. EGCG also forbids the elevated MMP-9 expression from osteoblasts induced by *P. gingivalis* extracts.³

It has also been concluded that though the benefits of green tea towards the periodontium maybe currently unclear, another important finding is that its consumption is in no way harmful towards periodontal health.

Green tea consumption has also been explored widely in the medical field and various studies have observed benefits such as reduced risk for mortality, type II diabetes, cardiovascular diseases, liver diseases, arthritis, stress and cancer. It also helps in reducing weight and also acts as an anti-aging drink.

Therefore, based on the findings of this study, we believe that further clinical trials of short and long term durations hold promise for assessing the clinical efficacy of green tea in the field of periodontics.

There are however a few drawbacks to the present study. The disc diffusion method used in this study is capable of measuring varying degrees of

antibacterial activity; however it is not possible to deduce Minimum Inhibitory Concentration or minimum bactericidal concentration¹¹. Hence the ideal concentration of green tea extract still remains unknown. Further analysis of the concentration of green tea in each cup would be required to assess if the results of this study or any other can be applied to an external population.

CONCLUSION:

Based on the results obtained from the present research it can be terminated that green tea extract, does indeed possess antimicrobial activity against the periodontal pathogens *P.gingivalis*, *P.intermedia* and *A.actinomycescomitans*. However future more precise clinical trials are required to know for its ideal concentration that inhibits periodontal pathogens and along with its activity on the periodontal biofilm.

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