ANTIBACTERIAL ACTIVITY OF GREEN TEA LEAVES AND LEAVES COMMONLY ADDED TO GREEN TEA PREPARATIONS, AGAINST COMMON PERIODONTAL PATHOGENS - AN IN VITRO STUDY

Authors:

Suneesh Kuruvilla[1]*
Ashbin Paul[2]
Ashique Manzoor[3]
Akhil Sasi[4]
Derin George[5]
Rohit Krishna[6]
Priya Babu[7]
Subramaniam R[8]
Jesline Merly James[9]

[1]Senior Lecturer
Department of Public Health Dentistry
Indira Gandhi Institute of Dental Sciences
Nellikuzhi P. O., Kothamangalam 686 691

[2,3,4,5,6]House Surgeon
Department of Public Health Dentistry
Indira Gandhi Institute of Dental Sciences
Nellikuzhi P. O., Kothamangalam 686 691

[7]Senior Lecturer
Department of Public Health Dentistry
Indira Gandhi Institute of Dental Sciences
Nellikuzhi P. O., Kothamangalam 686 691

[8]Professor and Head
Department of Public Health Dentistry
Indira Gandhi Institute of Dental Sciences
Nellikuzhi P. O., Kothamangalam 686 691

[9]Reader
Department of Public Health Dentistry
Indira Gandhi Institute of Dental Sciences
Nellikuzhi P. O., Kothamangalam 686 691

*Address for correspondence
Dr. Suneesh Kuruvilla, Senior Lecturer
Department of Public Health Dentistry
Indira Gandhi Institute of Dental Sciences
Nellikuzhi P. O., Kothamangalam 686 691
E mail: drsuneeshkuruvilla@gmail.com

ABSTRACT

Background: Periodontal disease, a chronic inflammatory disease resulting in progressive attachment and alveolar bone loss, is, after dental caries, one of the most important oral diseases contributing to the global burden of chronic disease. India is one of the largest consumers of tea. Green tea market has been steadily increasing in India due to the proven health benefits. Various studies have shown the beneficial effects of green tea on cardiovascular diseases, obesity and type 2 diabetes mellitus. The study was conducted with an objective of evaluating the in vitro antimicrobial activity of Camellia sinensis, Ocimum tenuiflorum, Mentha arvensis, Moringa olifera and Citrus limon leaves on Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans.

Methodology: The study was an in-vitro study. The fresh leaves of Camellia sinensis, Ocimum tenuiflorum, Mentha arvensis, Moringa olifera and Citrus limon were obtained and were sundried and powdered. Solvent extracts were prepared with methanol by maceration technique. Antibacterial activity was assessed using the agar well diffusion assay and minimum inhibitory concentration was assessed using the serial tube dilution technique.

Results: It was observed that the maximum zone of inhibition against Porphyromonas gingivalis was demonstrated by 0.2% chlorhexidine (positive control), followed by Moringa olifera and Camellia sinensis. Against Aggregatibacter actino mycetemcomitans, 0.2% chlorhexidine (positive control) demonstrated the maximum antibacterial activity, followed by Camellia sinensis. Moringa olifera.

Conclusion: The study showed that all the five extracts used in the study demonstrated antibacterial activity against both the periodontal pathogens.

Key words: Camellia sinensis, Ocimum tenuiflorum, Mentha arvensis, Moringa olifera, Citrus limon, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans.

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Background:

Infectious diseases are the third most significant reason of mortality around the world. The burden of infectious diseases is high in developing countries. This is because of the emergence of multi-drug resistant pathogens due to poor health-care facilities, and over-the-counter availability and misuse of antimicrobial agents.[1]

World Health Organization (WHO) has urged the search for new antimicrobial compounds and natural bioactive compounds which can be a good candidate in this perspective. The use of conventional antimicrobial agents against these infections is always associated with problems such as the development of multiple drug resistance and adverse side effects. In some cases, the use of synergistic antibiotic drug combination with bioactive compounds is the only option for the treatment of multi-drug resistant (MDR) bacteria.[1]

Periodontal disease, a chronic inflammatory disease resulting in progressive attachment and alveolar bone loss, is, after dental caries, one of the most important oral diseases contributing to the global burden of chronic disease, and it meets the criteria for consideration as a public health problem that requires action. Furthermore, multiple studies have reported association of periodontal disease with various systemic diseases and conditions, such as diabetes, HIV, atherosclerotic vascular disease, rheumatoid arthritis, adverse pregnancy outcomes, obesity, and metabolic syndrome. The costs of treating the periodontal disease are substantial and due to the current state of knowledge of the risk factors implicated in the etiopathogenesis of periodontal disease [e.g., smoking, alcohol, poor diet, lack of exercise, stress, distress, and psychological-coping resistance], there is sufficient information to allow the effective control of the common forms of the disease. As non-communicable chronic diseases share numerous risk factors with periodontal diseases, The Common Risk Factor Approach (CRFA) and the health promotion approach have been acknowledged as key aspects of the strategies aimed at those who are known to be at high risk.[2,3]

India is one of the largest consumers of tea. Green tea market has been steadily increasing in India due to the proven health benefits. Various studies have shown the beneficial effects of green tea (Camellia sinensis), not only on cardiovascular diseases but also on obesity and type 2 diabetes mellitus.[4] Leaves such as Ocimum tenuiflorum (Tulsi), Mentha arvensis (Mint), Moringa olifera leaves (Drum stick) and Citrus limon leaves (Lemon) leaves, have been added to the commercially available green tea (or fresh leaves are used while preparation of green tea), to enhance the medicinal benefits of the preparation.

The study was conducted with an objective of evaluating the in vitro antimicrobial activity of Camellia sinensis, Ocimum tenuiflorum, Mentha arvensis, Moringa olifera and Citrus limon leaves on Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans; and assessing the minimal inhibitory concentrations of the extracts against the two microorganisms.

METHODOLOGY

The study was an in-vitro study. The fresh leaves of Camellia sinensis, Ocimum tenuiflorum, Mentha arvensis, Moringa olifera and Citrus limon were obtained and were sundried and powdered using a mixer grinder. All specimens will be identified by a botanist for their authenticity.

Preparation of the extracts[5]

Solvent extracts were prepared with methanol by maceration technique. 10g of plant powder was thoroughly mixed with 100ml methanol for a period of 24 hours and the mixture thus obtained was filtered using a muslin cloth followed by Whatman's filter paper No.1. The filtrate thus obtained was concentrated by complete evaporation of solvent at room temperature to yield the pure extract. 100% pure solutions of crude extracts were prepared by mixing well the appropriate amount of dried extracts with an inert solvent di methyl sulfoxide (negative control).[5] Chlorhexidine (0.2%) was used as the positive control.[6]

Agar well diffusion assay

Petri dishes containing 18ml. of blood agar supplemented with haemin and vitamin K for the

periodontal pathogens were inoculated with approximately 100ml of respective microbial strain using swab technique. Wells of 8 mm diameter were cut into solidified agar media using a sterilized standard device. 100ml of each extract were poured in the respective well and the plates were incubated at 37°C for 48 hours.[5] To ensure the consistency of all findings, the experiment was performed and repeated under strict aseptic conditions. The antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by each extract at the end of incubation period.

Determination of Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of the extracts capable of inhibiting the growth of the bacterium tested. In the present study, MIC was determined using 'Serial tube dilution technique'.

In this technique the tubes of broth medium, containing graded doses of compounds were inoculated with the test organisms. After suitable incubation, each tube was assessed for visible growth in terms of turbidity. MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial strain using reflective viewer.[5]

Statistical analysis

The diameter of inhibition zone was calculated for each group, summarised and expressed as mean ± standard deviation. One-way ANOVA followed by Tukey's post hoc test was used for inferential analysis. p value < 0.05 was considered statistically significant.

RESULTS

The study showed that all the five extracts used in the study demonstrated antibacterial activity against both the periodontal pathogens and hence MIC was also assessed. The results zones of inhibition created by the test products as assessed by the agar well diffusion technique is shown in Table 1 and Table 2. The minimal inhibitory concentrations of each of the test products against both the periodontal pathogens, as seen with Serial tube dilution technique is outlined in Table 3.

It was observed that the maximum zone of inhibition against Porphyromonas gingivalis was demonstrated by 0.2% chlorhexidine (positive control), followed by Moringa olifera and Camellia Sinensis. Ocimum tenuiflorum and Mentha arvensis showed comparable antibacterial activity and Citrus limon showed the least activity against Porphyromonas gingivalis.

Sl. No.	Scientific Name	Common Name	Zone of inhibition	ANOVA and post hoc
1	Camellia Sinensis	Tea plant	18 ± 0.60	F = 558.34
2	Ocimum tenuiflorum	Holy Basil	16 ± 0.42	
3	Mentha arvensis	Wild mint	16 ± 0.22	6>4>1>2,3>5
4	Moringa olifera	Drumstick	20 ± 0.32	
5	Citrus limon	Lemon	9 ± 0.14	
6	0.2% Chlorhexidine	Positive control	22 ± 0.20	

Table 1: Antibacterial activity of the leaf extracts against Porphyromonas gingivalis

Against Aggregatibacter actinomycetemcomitans, 0.2% chlorhexidine (positive control) demonstrated the maximum antibacterial activity, followed by Camellia Sinensis. Moringa olifera and Mentha arvensis demonstrated comparable activity, followed by Ocimum tenuiflorum and Citrus limon.

It was observed that against Porphyromonas gingivalis, the least MIC was demonstrated by 0.2 % chlorhexidine, followed by Moringa olifera, Mentha arvensis, Ocimum tenuiflorum, Camellia sinensis and Citrus limon. Against Aggregatibacter actinomycetemcomitans, the least MIC was seen with Citrus limon, followed by Ocimum tenuiflorum, Mentha arvensis, Moringa olifera Camellia sinensis and 0.2% chlorhexidine.

DISCUSSION

The discovery and development of medicinal plants as drugs, especially from India and some African countries has proven effective in the treatment of multi-drug resistant patterns among clinical and environmental isolates. The primary benefits of using plant-derived medicines are that they are safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment.[1]

Various research conducted report the effective antimicrobial properties of Camellia sinensis[1], Ocimum tenuiflorum (Tulsi)[9], Mentha arvensis (Mint)[10], Moringa olifera leaves (Drum stick)[11] and Citrus limon[12] against various bacteria and fungi.

Sl. No.	Scientific Name	Common Name	Zone of inhibition	ANOVA and post hoc
1	Camella Sinensis	Tea plant	24 ± 0.45	F = 501.67
2	Ocimum tenuiflorum,	Holy Basil	20 ± 0.56	
3	Mentha arvensis,	Wild mint	22 ± 0.32	6>1>4,3>2>5
4	Moringa olifera	Drumstick	23 ± 0.21	
5	Citrus limon	Lemon	18 ± 0.19	
6	0.2% Chlorhexidine	Positive control	26 ± 0.19	

Table 2:
Antibacterial activity of the leaf extracts against Aggregatibacter actinomycetemcomitans

Sl. No.	Scientific Name	Common Name	P gingivalis (in µ/ml)	A actinomycet-emcomitans (in µ/ml)
1	Camella Sinensis	Tea plant	64	24
2	Ocimum tenuiflorum,	Holy Basil	16	20
3	Mentha arvensis,	Wild mint	4	20
4	Moringa olifera	Drumstick	2	23
5	Citrus limon	Lemon	128	18
6	0.2% Chlorhexidine	Positive control	<1	26

Table 3:
Minimal inhibitory concentrations of the leaf extracts against the periodontal pathogens

Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans are among the most common pathogens responsible for periodontal infections.[13] Studies also indicate that the adverse cardiovascular effects from periodontal diseases are due to a few putative or high-risk bacteria including P. gingivalis and A. actinomycetemcomitans. They have also been identified as a contributory cause to atherosclerosis.[14]

It is considered that the ideal antibiotic used in the management of periodontal disease should be pathogen specific, allogenic and nontoxic, substantive, and it should not be in general use for treatment of other diseases, as well as inexpensive. Currently, an ideal such antibiotic does not exist. Although oral bacteria are susceptible to many antibiotics, no single antibiotic at concentrations achieved in body fluids inhibits all putative periodontal pathogens. The present antimicrobials indicted for periodontal infections include Tetracycline, doxycycline, metronidazole, amoxycillin, ciprofloxacin, macrolides and local application of chlorhexidine.[15]

This research focuses on the potential use of leaves that are commonly available in household, that are used together with green tea leaves in preparation of a healthy drink. Periodontal diseases being considered a public health problem globally. This research demonstrates antimicrobial activity of all the leaf extracts against the periodontal pathogens. The use of these additives with the green tea can be an intervention, targeting the 'Common Risk Factor Approach' for a healthier lifestyle, without a need for a behavioural change. Furthermore, this research can pave way to further studies with broader scope, on utilizing the antimicrobial properties towards development of newer local and systemic drug delivery systems for the treatment of periodontal disease and its impacts on the health and well-being. This can possibly contribute to the various efforts in tackling the emerging antimicrobial resistance.

The results of zones of inhibition and minimum inhibitory concentration indicate an innovation of using the leaf extracts in various forms of drug delivery, to extract its potential therapeutic benefits.

The study is a preliminary investigation to assess the antibacterial efficacy of the leaf extracts that are commonly used in tea preparations. Further research in identifying the active ingredient is needed to tap the potential of these extracts and enhancing the pharmaceutical application. Further in vivo studies and clinical trials are needed to substantiate the application of the extracts topically and systemically. Most of these leaves are readily available plant products.

CONCLUSION

The green tea leaf extract Camellia sinensis and extracts of leaves commonly added to green tea preparations, namely Moringa olifera, Ocimum tenuiflorum, Citrus limon and Mentha arvensis demonstrated antibacterial activity against Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans. Against Porphyromonas gingivalis, maximum zone of inhibition against was demonstrated by Moringa olifera and Camellia sinensis followed by Ocimum tenuiflorum, Mentha arvensis and Citrus limon. Against Aggregatibacter actinomycetemcomitans, maximum antibacterial activity, was demonstrated by Camellia Sinensis, followed by Moringa olifera, Mentha arvensis, Ocimum tenuiflorum and Citrus limon.

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