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ANTIMICROBIAL EFFECT OF GARCINIA INDICA ON PERIOPATHOGENS - AN IN VITRO STUDY

Periodontics

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ABSTRACT

Background: Gingivitis is a chronic inflammatory disease that is caused by dental plaque. A vast number of microorganisms are responsible for the formation of plaque.

Garcinia indica is distributed in the tropical regions and is an abundant source of hydroxycitric acid, garcinol, anthocyanins which has an anti oxidant, anti inflammatory and anti microbial properties.

Aim: To evaluate the efficacy of Garcinia indica fruit rind extract as an antimicrobial agent against various periopathogens.

Materials And Methods: The bacterial strains of S. mitis, S. salivarius and P gingivalis were selected and cultured in Brain Heart Infusion (BHI) Broth. The antimicrobial property of Garcinia indica was determined by performing minimum inhibitory concentration (MIC), minimum Bactericidal Concentration (MBC) and zone of inhibition.

Results: Garcinia indica inhibited the growth of Streptococcus mitis, Streptococcus salivarius and Porphyromonas gingivalis in a dose dependent manner.

Conclusion: Result of our study shows that Garcinia indica has antibacterial property against periopathogens and could be used as an adjunct for prevention of periodontal disease caused by these pathogens.

KEYWORDS

Garcinia indica, Kokum, Gingivitis, Minimum inhibitory concentration, Minimum bactericidal concentration.

INTRODUCTION

Dental Plaque is one of the foremost factors that induces gingivitis. Gingivitis is a chronic inflammatory disease that mainly affects gingiva with no associated alveolar bone loss or attachment loss.[1] Various colonies of microorganisms helps in genesis of plaque. Few of the primary colonizers of dental Plaque S. mitis and S. salivarius[2] whereas, P. gingivalis a secondary colonizer in the dental biofilm may be responsible for the progression of the disease.[3]

Commonly, chlorhexidine mouthwash used as an adjunct to SRP has some of the disadvantages such as irritation and staining.[4] Recently herbal and certain natural fruit extracts are gaining popularity to be used as mouth washes as they have very few side effects if any.

Garcinia indica frequently known as kokum is found in numerous topical regions.[5] Important active compounds present in fruit rind of Garcinia indica are hydroxycitric acid, garcinol, anthocyanins, isogarcinol, citric acid and polyphenols[6] and is acknowledged for it's anti-obesity, anticancer, anti-diabetic, antioxidant and antimicrobial properties.[7]

However, there has been an inadequate data in the literature on efficacy of Garcinia indica on Periopathogens.

MATERIALS AND METHODS

This Study was planned and designed to evaluate the antimicrobial effect of Garcinia indica in the Department of Periodontics, PMNM Dental College and Hospital, Bagalkot and the laboratory procedures were processed in the Department of Molecular and Microbiology, Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences & Research Centre, Belgaum (India).

Garcinia indica was taken as the test agent in this study and was evaluated for minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and Disc diffusion test .

MIC Procedure For S.mitis, S.salivarius And P.gingivalis

9 distinct dilutions of Garcinia indica extract was prepared for each concentration. In initial test tube 20µl of Garcinia indica was added into the 380µl of Thioglycollate broth. To obtain dilutions, 200l of thioglycollate broth was added in all 9 test tubes individually. Subsequently, to the first tube containing 200µl of thioglycollate broth, 200µl of solution was added from the initial test tube and thus 10^{-1} dilution was obtained. For individual concentration of Garcinia indica the serial dilution was repeated up to 10^{-9} dilution. Out of the preserved stock cultures of requisite organisms, 5µl was abstracted and added into 2ml of thioglycollate broth. 200µl of preceding culture suspension was added, for each of the serially diluted tube. To obtain the results, that was in the form of turbidity, all the tubes were incubated in anaerobic jar at 37°C for 48-72 hour.

MBC Procedure For S.mitis, S.salivarius And P.gingivalis

Out of the MIC dilutions tubes, first 3-5 tubes which were found to be sensitive for MIC test were plated and then incubated for 24 hrs. Followed by which the colony count was taken into account for MBC test. On condition that, there is no growth then - its bactericidal effect and if there is growth then - its bacteriostatic effect.

Disc Diffusion Test For S.mitis, S. salivarius and P.gingivalis

Brain Heart Infusion agar was used as a culture media for the disc diffusion test. A loop or swab was used to transfer the colonies to the plates.

For stock solution, 10mg of Garcinia indica was taken and was dissolved in 1ml of DMSO (Dimethylsulfoxide). Five different wells were prepared in agar plate. Using micropipette 75µl, 50µl, 25µl, 10µl and 5µl was added in each well and then the plates were incubated within 15 min of compound application. Plates were turned upside down, and stacked for 18-24 hrs and was incubated at 37 ºC. To compute the diameter, inhibition zone was estimated to nearby whole millimeter by holding the measuring device.

RESULT

Table 1 Shows The MIC Results Of Garcinia Indica Extract Against Few Of The Periopathogens.

Table 1- MIC of Garcinia indica at different concentrations against S. mitis, S. salivarius and P. gingivalis.

Sl. No.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
	Kokum Powder										
	S. mitis										
01	1%	S	S	S	S	R	R	R	R	R	R
02	3%	S	S	S	R	R	R	R	R	R	R
03	5%	S	S	S	R	R	R	R	R	R	R
04	10%	S	S	S	R	R	R	R	R	R	R
	S.salivarious										
01	1%	S	S	R	R	R	R	R	R	R	R
02	3%	S	S	R	R	R	R	R	R	R	R
03	5%	S	R	R	R	R	R	R	R	R	R
04	10%	S	R	R	R	R	R	R	R	R	R
	P. gingivalis										
01	1%	S	S	R	R	R	R	R	R	R	R
02	3%	S	S	R	R	R	R	R	R	R	R
03	5%	S	S	S	R	R	R	R	R	R	R
04	10%	S	S	S	S	R	R	R	R	R	R

S: Sensitive, R:Resistant and MIC: Minimum inhibitory concentration.

MIC for 1% concentration of Garcinia indica extract against S. mitis was found to be 12.5µg/ml where as 3%, 5% and 10% concentration of the extract MIC was 25µg/ml S. salivarius was resistant till 25µg/ml for both the concentrations i.e 1% and 3%, so MIC value was 50µg/ml and for 5% and 10% it was resistant till 50µg/ml, MIC value being 100µg/ml. When tested against P. ginigivalis MIC value was 50µg/ml where as MIC value for 5% was 25µg/ml and for 10% it was 12.5µg/ml.

MBC results of Garcinia indica extract against Periopathogens. When tested against S.mitis, no bacterial growth was seen for 1% at 12.5µg/ml and for 3%, 5% and 10% at 25µg/ml. S. salivarius did not show any growth for 1% and 3% at 50µg/ml for 5% and 10% at 50µg/ml. When tested against P. gingivalis there was no bacterial growth for 1% and 3% at 50µg/ml , for 5% at 25µg/ml and for 10% at 12.5µg/ml.

Disc diffusion results are shown in Table no. 2. At 10% Garcinia indica showed highest inhibition zone of 20mm in P.gingivalis followed by S. mitis of 15mm at and least for S. salivarius of 10mm.

Table 2- Zone of inhibition at different concentrations against S. mitis, S. salivarius and P. gingivalis.

Sl. No.	Samples	1%	3%	5%	10%
	Kokum Powder				
01	*S. mitis*	R	R	12mm	15mm
02	*S.salivarius*	R	R	R	10mm
03	*Pg*	12mm	15mm	18mm	20mm

R:Resistant

DISCUSSION

Gingivitis is a most common prevailing periodontal disease, initiated due to the accumulation of dental plaque. The microflora of dental plaque associated with gingivitis consists of initial colonizers (S. mitis, S. salivarius) and P. gingivalis as secondary colonizer. For the treatment of gingivitis most commonly chlorhexidine mouth wash is being used as an adjunct to SRP, which is associated with certain side effects like irritation and staining.[4] Anthocyanins are pigmented flavonoids found in Garcinia indica, that exhibit strong antioxidant property by preventing oxidation of ascorbic acid, scavenging free radicals and inhibits oxidative enzymes.[8] Burdulis D et al[9] showed that anthocyanin tends to exhibit antimicrobial and anti-inflammatory activity. Garcinol is also known to have antioxidant property it interferes with two enzymes, 5- lipoxygenase and prostaglandin synthase which has principal role in inflammation. Isogarcinol posses similar actions as of garcinol and has been affirmed to have anti-inflammtory properties.[8] All of these constituents of garcinia indica play in harmony to bring about antimicrobial as well as an anti-inflammatory effect. Gram positive streptococci such as S. mitis and S. salivarius were chosen for the present study as they are among few of the initial colonizers of dental plaque biofilm and helps in formation of dental plaque.[10] P. gingivalis which is a gram negative bacteria, is a key stone pathogen and also a secondary colonizer that is present in dental plaque and plays a key role in periodontal destruction.[11] Results of this study showed that although Garcina indica 1% and 3% was effective against S. mitis when compared to S. salivarius and P. gingivalis. 5% and 10% concentrations of Garcinia indica showed better efficacy against all the test pathogens. The MBC values were found to be almost double of the MIC values. Zone of inhibition of Garcinia indica was least for S. salivarius (10mm), followed by S. mitis (15mm) and highest in case of P. gingivalis (20mm) at 10% concentration. Widyarman AS et al.[12] demonstrated that Garcinia mangostana peel extract was used, which belongs to the same family as Garcinia indica and was found to be efficacious in inhibiting S. mutans and P. gingivalis biofilms. Our study has shown that Garcinia indica extract is effective against S. mitis, S. salivarius and P. ginigivalis with different concentrations. At 5% and 10% concentration MIC, MBC and zone of inhibition for S. mitis, S. salivarious and P. gingivalis was better.

CONCLUSION:

The results of this present study showed that different concentrations of Garcinia indica were effective against strains of S. mitis, S. salivarius and P. gingivalis. Therefore, it can be hypothesized from this study that appropriate formulation of Garcinia indica can be used as an adjunct to prevent periodontal disease. Hence, further clinical studies can be taken up in supplementation of this in vitro study.

REFERENCES

1. Salgado AD, Maia JL, Pereira SL, de Lemos TL, Mota OM. Antiplaque and antigingivitis effect of a gel containing Punica granatum Linn extract: a doubleblinded clinical study in humans. J Appl Oral Sci. 2006;14:162-6.
2. Buommino E, Scognamiglio M, Donnarumma G, et al. Recent advances in natural product-based anti-biofilm approaches to control infections. Mini Reviews Med Chem 2014; 14:1169-82.
3. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25:134-44.
4. Florta L, Gjermo P, Rolla G, Waerhaug J. Side effects of chlorhexidine mouth washes. Scand J Dent Res. 1971;79:119-25.
5. Chandran SMD Conservation and sustainable use of cultivated and wild tropical diversity: Introduction to good practices. Karnataka Journal of Agricultural Science. 2009;22(1): 244-5.
6. Parasharami VA, Kunder GG and Desai N. Recent pharmacological advances of endangered species of south India : Garcinia indica choisy. J. Sci. Res 2015;8(5)1-10.
7. Hemshekhar M, Sunitha K, Santhosh MS, Devaraja S, Kemparaju K, Vishwanath BS, Niranjana SR, Girish KS. An overview on genus Garcinia: phytochemical and therapeutical aspects. Phytochem Rev. 2011;10: 325-51.
8. Bridle P and Timberlake CF. Anthocyanins as natural food colour-selected aspects. Food Chemistry. 1997;58(1-2): 103-9.
9. Burdulis D., Sarkinas A, Justin I, Stackevicence. Comparative study of anthocyanin composition, antimicrobial and antioxidant activity in bilberry and blueberry. Fruits Acta Pol. Pharm. 2009;66:399-408.
10. Kolenbrander PE. Oral microbial communities: Biofilms, interactions, and genetic systems. Annu Rev Microbiol 2000; 54: 413–37.
11. Mysak J, Podzimek S, Sommerova P, Lyuya-Mi Y, Bartova J, Janatova T et al. Porphyromonas gingivalis: major periodontopathic pathogen overview. J. Immunol. Res. 2014;476068.
12. Widyarman AS, Lay SH, Wendhita IP, Tjakra EE , Murdono FI , Binartha CTO. Indonesian Mangosteen Fruit Peel Extract Inhibits S. mutans and P. gingivalis in Biofilms In vitro. Contemp Clin Dent. 2019;10(1):123-8.