



## ANTIMICROBIAL ACTIVITY OF GUAVA LEAF EXTRACT ON PERIODONTAL PATHOGENS

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### ABSTRACT

**Background:** *P.gingivalis*, *P.intermedia* and *A.actinomycetemcomitans* are strongly associated with chronic periodontitis. Guava leaf extract is a proven antibacterial agent for treating systemic bacterial infections. This study aims at evaluating Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of guava leaf extract required against periodontal pathogens.

**Material and Methods:** For MIC, serial dilutions of extract were made in culture tubes and the periodontal pathogens were added to observe turbidity. For MBC, the same culture tubes were inoculated and incubated to observe for growth.

**Results:** MIC test showed that *P.gingivalis* and *P.intermedia* got inhibited at 0.4 µg/ml concentration while *A.actinomycetemcomitans* was inhibited at 1.6 µg/ml. MBC test showed that the extract was bactericidal to only *P.intermedia* which was at 12.5 µg/ml.

**Conclusion:** Guava leaf extract is effective against periodontal pathogens and can be used to treat periodontal diseases.

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## INTRODUCTION

Periodontal diseases are infectious diseases with bacterial origin present in dental plaque. Periodontitis is a complex disease in which disease expression involves intricate interactions of the biofilm with the host inflammatory response and subsequent alterations in bone and connective tissue metabolism [Kornman, 2008]. So the treatment for periodontal diseases focuses mainly on elimination of periodontal pathogens, which includes mechanical debridement and use of chemotherapeutic agents. In an attempt to overcome the limitations of mechanical debridement and ill effects of chemotherapeutic agents, research still continues to find new and better alternatives to conventional treatment protocols. One of the alternatives are the medicinal herbs which has been extensively used in ayurveda but lacks sufficient research in modern medicine. Various herbs like Aloe vera, Curcumin, calendula and others are being extensively researched to treat periodontal diseases. Guava is one of them which has been used to treat systemic diseases but lacks research in periodontal medicine.

Botanically known as *Psidium guajava* and commonly guava, it has been used for systemic diseases owing to its antidiarrheal, antimicrobial, antiparasitic, antitussive, hepatoprotective, antioxidant, antigenotoxic, antimutagenic,

antiallergic, anticancer and anti-hyperglycemic and analgesic properties. The leaves contain two important flavonoids, quercetin having spasmolytic, antioxidant, antimicrobial, anti-inflammatory actions and guajaverin which has antibacterial action. [Ravi and Divyashree, 2014]

### Antimicrobial activity of guava leaf extract

It has shown inhibitory effects against *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Bacillus cereus*, *Proteus* spp., *Shigella* spp. and *Escherichia coli* which causes intestinal infections. [Chah et al, 2006]. Recent studies have proven antifungal and antibacterial properties against *E.coli*, *Salmonella typhi* and *Klebsiella pneumonia* [Dhiman et al, 2011a]. Its anticariogenic activity has been tested in comparison to chlorhexidine which showed inhibitory effect against *L.acidophilus*. [Jain et al, 2014a]. Extracts from roots of guava is also effective against *L.acidophilus*. The cytolytic and pro-inflammatory responses of human leukocytes exposed to *A. actinomycetemcomitans* leukotoxin was completely eliminated by the extract. [Kwamin et al, 2012]. Taking into consideration the broad spectrum antibacterial activity of guava leaf extract, this study was done to evaluate minimal concentration required to inhibit the growth of common periodontal pathogens.

### Antimicrobial susceptibility of a drug

To check for sensitivity of a bacteria, it is necessary to test isolated pathogens against appropriate antimicrobial agents. The lowest concentration of an antibiotic that will inhibit the

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growth of the organism being tested is known as the minimal inhibitory concentration (MIC). It determines the concentration of antibiotic needed to inhibit the pathogen. The minimal bactericidal concentration (MBC) is defined as the lowest concentration of an antibiotic killing the majority (99.9%) of a bacterial inoculum. [Schwalbe and Moore, 2007].

**Aim**

To evaluate Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of guava leaf extract against *P.gingivalis*, *P.intermedia* and *A.actinomycetemcomitans*.

**MATERIAL AND METHODS**

The guava leaf extract was obtained from a local dealer under aseptic conditions. Alcoholic extract of the leaves was taken as alcoholic extract and not distilled water showed antibacterial activity against two gram-positive bacteria, *Staphylococcus aureus* and *Bacillus cereus*. [Dhimanet al,2011b]

**Determination of MIC**

The traditional method of determining the MIC is with the broth dilution technique, where serial dilutions of antibiotic are incorporated into the broth media in either the wells of microtiter plates or in culture tubes. For MIC of guava leaf extract, nine dilutions of extract were done with brain heart infusion broth (BHI). In the initial tube 20 microliter of extract was added into 380 microliter of BHI broth. 200 microliter of BHI broth was added into the next 9 tubes separately for dilutions. Then from the initial tube, 200 microliter was transferred to the first tube containing 200 microliter of BHI broth. This was considered as 10<sup>-1</sup> dilution. From 10<sup>-1</sup> diluted tube 200 microliter was transferred to second tube to make 10<sup>-2</sup> dilution. The serial dilution was repeated up to 10<sup>-9</sup> dilution. From the maintained stock cultures of *P.gingivalis*, *P.intermedia* and *A.actinomycetemcomitans*, 5 microliter was taken and added into 2ml of BHI (brain heart infusion) broth. In each serially diluted tube 200 microliter of above culture suspension was added. The tubes were incubated at 37°C for 48-72 hrs in CO<sub>2</sub> jar and observed for turbidity.

**Determination of MBC**

MBC was done to see whether there was bacteriostatic or bactericidal effect of the extract against the organisms. The MIC dilutions tubes were plated and incubated for 24 hrs in anaerobic conditions. The next day colony count was taken. If there is no growth then it has bactericidal effect and if there is growth then it is bacteriostatic.

**RESULTS**

MIC test showed that *P.gingivalis* and *P.intermedia* got inhibited at 0.4 µg/ml concentration while *A.actinomycetemcomitans* was inhibited at 1.6 µg/ml. MBC test showed that the extract was bactericidal to only *P.intermedia* which was at 12.5 µg/ml. The results for MIC and MBC test are given in table 1 and table 2 respectively.

**Table 1** result of serial dilutions to test for MIC.

Extract concentration	100µg/ml	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
<i>P.gingivalis</i>	S	S	S	S	S	S	S	S	S	R
<i>P.intermedia</i>	S	S	S	S	S	S	S	S	S	R
<i>A.actinomycetemcomitans</i>	S	S	S	S	S	S	S	R	R	R

S- sensitive R-resistant

**Table 2** Result of inoculation of MIC tubes to test for MBC.

Extract concentration	100µg/ml	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
<i>P.gingivalis</i>	28	30	34	36	40	54	60	67	80	89
<i>P.intermedia</i>	NG	NG	NG	NG	04	04	10	11	15	26
<i>A.actinomycetemcomitans</i>	30	34	39	42	58	75	82	96	102	113

NG- no growth

**DISCUSSION**

Using plant extracts as a chemotherapeutic agents gives an edge over conventional synthetic drugs if supported with scientific evidence. These are found to be useful in dentistry due to its antibacterial, antioxidant, immune modulatory and analgesic actions. Present study focussed on unexplored benefits of guava leaf extract in eliminating periodontal pathogens. Guava leaves contains essential oils, flavonoids, saponins, nerolidiol, β-sitosterol, ursolic, crategolic and guavavolic acid which is responsible for its antibacterial action [Jain et al, 2014b]. *P. guajava* is also effective against *Pseudomonas lundensis*, *Aspergillus niger*, and *Aspergillus flavus* [Hema et al., 2009]. *Streptococcus mutans*, *Streptococcus sanguinis* and *Streptococcus salivarius*, which are primary plaque colonizers were inhibited by guava leaf extract which was comparable to chlorhexidine. [Chandrashekar et al, 2014]

The predisposition of periodontal diseases is multifactorial but the primary causative agent is dental plaque which harbours multi-species of microorganisms. Amongst, those species which are most important are *P.gingivalis*, *P.intermedia* and *A.actinomycetemcomitans*. So the treatment for periodontal diseases aims at reduction and inhibition of these pathogens by both mechanical debridement and chemotherapeutic agents. So this study was done to evaluate Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) against *P.gingivalis*, *P.intermedia* and *A.actinomycetemcomitans* against guava leaf extract.

An in-vitro study proved that guava leaf extract was effective against *P.intermedia*. Also, leukotoxin (LtxA) of *A.actinomycetemcomitans* was significantly reduced by guava extract [Toma and Genet [Internet][Dissertation] 2014]. Both these studies were in accordance to our study which showed that all the three periodontal pathogens tested got inhibited while only *P.intermedia* was killed by the extract.

**CONCLUSION**

This study generates evidence with regards to usage of ayurveda in dentistry. From the study we can conclude that guava leaf extract has the potential to act against periodontal pathogens. The present study can be further extended against other species of pathogens. The values of MIC and MBC from this study can be used to formulate medications for treatment of periodontal diseases. In this era where resistance of bacteria to synthetic alternatives is a major problem, guava leaf extract can be further studied for its anti-inflammatory and immune-modulatory properties so that it can be brought into mainstream of drugs to treat periodontal diseases.

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