

Antibacterial activity of curcumin (turmeric) against periopathogens - An *in vitro* evaluation

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Abstract

Background: Chronic periodontitis, one of the main causes of tooth loss, is an inflammatory disease caused by periodontopathic bacteria in periodontal tissues. Among them, the red complex consisting of *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* is considered to be important consortium in the progression of periodontitis. Since curcumin has antimicrobial, antioxidant, astringent, and other useful properties, pharmacological action and its therapeutic role are quite useful in dentistry also.

Aim: The aim of this study was to examine the antibacterial potential of curcumin, against standard strains of common periopathogens.

Materials and Methods: The bacterial strains, namely, *P. gingivalis* (ATCC 33277), *Prevotella intermedia* (ATCC 25611), and *T. forsythia* (ATCC 43037) were cultured on thioglycolate broth, and *Aggregatibacter actinomycetemcomitans* (ATCC 29523) was cultured on Brain Heart Infusion Broth. Minimum inhibitory concentration was determined, by serial broth dilution of curcumin to 100, 50, 25, 12.5, 6.5, 3.12, 1.6, 0.8, 0.4, and 0.2 mg/ml, respectively. The tubes were then incubated for 48 h at 37°C.

Results: Curcumin impeded the growth of *P. gingivalis*, *P. intermedia*, *T. forsythia*, and *A. actinomycetemcomitans* in a dose-dependent manner and suppressed completely at low concentrations of curcumin.

Conclusion: Curcumin has a property of antibacterial activity against periodontopathic bacteria and may be effective agent for preventing periodontal diseases.

Introduction

Periodontitis is an inflammation of supporting tissues of the teeth. It is usually a progressively destructive change, leading to loss of bone, periodontal ligament, and extension of inflammation from gingival to adjacent bone and ligament.^[1]

Periodontal diseases are referred to as inflammatory processes in periodontal tissues in response to the aggregation of bacteria on the teeth. The aggregation rarely leads to evident infection; however, the inflammatory response caused in the gingival tissue is responsible for the gradual loss of dental collagen's attachment to the alveolar bone. Left overlooked, this phenomenon might end in increased dental mobility, and eventually edentulism.^[2] Production of destructive metabolites by Gram-negative and positive bacteria of the microbial plaque in the oral cavity causes gingivitis, leading to the progression of inflammation to periodontal diseases.^[3]

Chronic periodontitis being the cause of the tooth loss is an inflammatory disease preceded by periodontopathic bacteria in periodontal tissues.^[4] Most of red complex bacteria such as *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* is considered to be an important consortium in the progression of periodontitis.^[2]

In our day-to-day life, mechanical and chemical plaque controls are important and effective methods to prevent periodontal disease. According to research, chlorhexidine, an antibacterial agent is the gold standard in today's practice but has concerning side effects.

In recent, the management approaches which include scaling, and root planning, periodontal surgical procedures along with adjunctive antibiotic therapy which reduces the clinical symptoms, elimination of periodontal pathogens and regeneration of beneficial bacterial flora. Not all patients or all sites respond uniformly and favorably to conventional

mechanical therapy.^[5] Focusing on these treatment options which possess limited effectiveness in high-risk populations and in those with advanced periodontal disease,^[6] and associated with adverse side-effects and antibiotic resistance,^[7] people are opting for alternative therapeutic and preventative measures which are safe, effective, and free of side effects.^[8]

Increasing popularity of traditional medicine has led researchers to investigate the adjunctive methods that use nutrients and functional foods to maintain the health status of periodontal tissues; for example, extracts of plants, including mastic exudated from *Pistacia lentiscus*^[9] and macrocarpals from *Eucalyptus globulus*,^[10,11] have also been investigated for their effects on periodontal health, particularly because of their safety.

Curcumin is the most potential substance in traditional medicine, in modern medicine.^[12] The main constituent of turmeric is known as curcumin. Curcumin is a yellow, water-insoluble pigment extracted from root of turmeric, which is used as spice and food-coloring agent in Southeast Asian cuisines.^[13] The components of turmeric are curcuminoids, which are mainly curcumin (diferuloylmethane), demethoxycurcumin, and bis-demethoxycurcumin. In ayurvedic medicine, curcumin acts as a “detoxifier of the body.” Today, science has documented several diseased conditions that can be healed by the active ingredients of turmeric. This study article wishes to quote from the Legacy of Charaka that captures the true spirit of Ayurveda and its vision of rigor: “Ayurveda owes its call not only to selfish goals or to worldly pleasure but also to compassion for fellow beings. In seeking to know my legacy, you have but seen the leaves of a universal tree, too vast for your eyes. May your sight grow and your quest never ends.”^[14]

The biological characteristics of Curcumin were scientifically reported in the mid-20th century. Schraufstatter and Bernt (1949) published in nature proved that curcumin is a biologically active compound having antibacterial properties.

Consider moving this to be part of the paragraph about the history of curcumin.

Thousands of years, curcumin used in ayurveda acts as anti-inflammatory,^[15,16] antioxidant,^[17,18] and anticancer^[19,20] effects which have been studied and also reported to have antibacterial and antifungal properties.^[21]

In oral flora, curcumin inhibited sortase A of *Streptococcus mutans*, which is a major cariogenic bacteria, capable of reducing *S. mutans* biofilm formation.^[22]

Curcumin has shown antibacterial property against a number of Gram-positive and Gram-negative bacteria (Negi, 1999).

In this study, we evaluated the antibacterial effects of curcumin on the growth of periodontopathic bacteria, such as *P. gingivalis* (ATCC 33277), *Prevotella intermedia* (ATCC 25611), *Aggregatibacter actinomycetemcomitans* (ATCC 29523), and *T. forsythia* (ATCC 43037).

Materials and Methods

This study was conducted at the Department of Molecular and Microbiology, Maratha Mandal's Nathajirao G. Halgekar

Institute of Dental Sciences & Research Centre, Belgaum (India).

The test agent in this study was curcumin. Stock solution of the test agent curcumin was made up in (dimethyl sulfoxide; Merck, Germany) to ensure complete solubilization. The periodontopathic bacteria were cultured in media with various curcumin concentrations and measured after 48 h. The bacterial strains, namely, *P. gingivalis* (ATCC 33277) a Gram-negative bacterium which is non-motile obligatory anaerobic rod, asaccharolytic, and colonies are black-pigmented formed on blood agar plates and require iron for its growth. *P. intermedia* (ATCC 25611) is Gram-negative, non-motile, obligatory anaerobes, single cells thrive in anaerobic growth conditions. *T. forsythia* (ATCC 43037) is an anaerobic, Gram-negative bacterial species in red complex in *Cytophaga-bacteroidetes* family which has been implicated in periodontal diseases.^[2] *A. actinomycetemcomitans* (ATCC 29523) is an immobile microaerophilic, facultative anaerobic, Gram-negative coccoid rod, strongly associated with pathogenesis of periodontal diseases cultured in Brain Heart Infusion (BHI) Broth. The test was assessed with minimum inhibitory concentration (MIC).

MIC procedure for *A. actinomycetemcomitans*

1. 9 dilutions of each drug have to be done with BHI for MIC.
2. In the initial tube, 20 μ L of drug was added into the 380 μ L of BHI broth.
3. For dilutions, 200 μ L of BHI broth was added into the next 9 tubes separately.
4. Then, from the initial tube, 200 μ L was transferred to the first tube containing 200 μ L of BHI broth. This was considered as 10^{-1} dilution.
5. From 10^{-1} diluted tube, 200 μ L was transferred to the second tube to make 10^{-2} dilution.
6. The serial dilution was repeated up to 10^{-9} dilution for each drug.
7. From the maintained stock cultures of required organisms, 5 μ L was taken and added into 2 ml of BHI broth.
8. In each serially diluted tube, 200 μ L of above culture suspension was added.
9. The tubes were incubated for 24 h and observed for turbidity.

MIC procedure for *P. gingivalis*, *P. intermedia*, and *T. forsythia*

1. 9 dilutions of each drug have to be done with thioglycollate broth for MIC.
2. In the initial tube, 20 μ L of drug was added into the 380 μ L of thioglycollate broth.
3. For dilutions, 200 μ L of thioglycollate broth was added into the next 9 tubes separately.
4. Then, from the initial tube, 200 μ L was transferred to the first tube containing 200 μ L of thioglycollate broth. This was considered as 10^{-1} dilution.
5. From 10^{-1} diluted tube, 200 μ L was transferred to the second tube to make 10^{-2} dilution.
6. The serial dilution was repeated up to 10^{-9} dilution for each drug.

7. From the maintained stock cultures of required organisms, 5 μ L was taken and added into 2 ml of thioglycollate broth.
8. In each serially diluted tube, 200 μ L of above culture suspension was added.
9. The tubes were incubated for 48–72 h in anaerobic jar at 37°C and observed for turbidity.

For facultative anaerobes, tubes were incubated at 37°C for 48–72 h in CO_2 Jar.

For strict anaerobes, tubes were incubated in anaerobic jars for 48–72 h.

Determination of MIC by broth diffusion method

MIC is the smallest range of product dilution capable of preventing bacterial growth with formation of inhibition zones measured in millimeters with a digital caliper. The MIC is the lowest concentration of antimicrobial agents that completely visually inhibits the growth of the microorganisms.

In the first MIC tube containing 100 μ L/ml broth, 100 μ L/ml of stock was added. After mixing, 100 μ L/ml was transferred to the second MIC tube. This was continued until the last (10th) tube. From the last tube, 100 μ L/ml of the final solution was discarded. By following this serial dilution, the concentrations of the aqueous extract achieved were as follows: MIC was determined, by serial broth dilution of curcumin to 100, 50, 25, 12.5, 6.5, 3.12, 1.6, 0.8, 0.4, and 0.2 μ L/ml, respectively.

To each of the ten such prepared MIC tubes with varying concentrations, 100 μ L of the earlier prepared strain of organism was added such that the final volume per tube was 200 μ L. The tubes were then incubated for 48–72 h at 37°C. After the incubation, the MIC values were determined by visual inspection of the tubes. In each series of tubes, the last tube with clear supernatant was considered to be without any growth and taken as MIC value. Turbidity in the MIC tube indicated growth of the bacteria implying that the bacteria are resistant to curcumin.

Statistical analysis

The present study is just simple and straightforward. The aim (null hypothesis) is to test curcumin against standard strains of common periopathogenes; hence, no statistical intervention is needed to draw any sensible conclusion. The results of the study are being shown Table 1. It is seen from Table 1 that the curcumin is sensitive for four strains given in 100 μ g/ml. In Aa, reduced doses of curcumin as small as 12.5 μ g/ml (40%) result in the same situation. For strains, it is effective up to 25 μ g/ml showing 30%. Therefore, it was concluded that the curcumin is effective in controlling pathogens of Aa and its type. However, the detailed study with other agents may be tried in the future, undertaken as clinical trials.

Results

The MIC of curcumin against tested periodontopathic bacteria is shown in Table 1.

Table 1: MIC of curcumin against Aa, Pg, Pi, and Tf

Turmeric powder	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
Aa	S	S	S	S	R	R	R	R	R	R
Pg	S	R	R	R	R	R	R	R	R	R
Pi	S	R	R	R	R	R	R	R	R	R
Tf	S	S	S	R	R	R	R	R	R	R

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

The results were triplicated. *A. actinomycetemcomitans* was suppressed almost completely at a very low concentration of 12.5 μ g/ml by curcumin, *P. gingivalis* and *P. intermedia* were suppressed at 100 μ g/ml. *T. forsythia* was inhibited at a concentration of 25 μ L/ml.

Discussion

Taking into consideration, the developing natural antibacterial agents against multidrug-resistant strains, this study on antibacterial activity of curcumin is valuable. The main purpose of this study was to evaluate the antibacterial effects of curcumin on periodontopathic bacteria. At higher concentrations, mostly all the pathogens are sensitive to curcumin, so the serial dilution method is used in this study to see the lowest concentrations that curcumin periopathogens are sensitive.

In this study MIC, curcumin showed its antibacterial effect of *A. actinomycetemcomitans* which was reported to be sensitive at lower concentration 12.5 μ g/ml and 25 μ g/ml *T. forsythia*, while *P. gingivalis* and *P. intermedia* were sensitive at higher concentration 100 μ g/ml. This indicated that curcumin strongly inhibited the growth of *A. actinomycetemcomitans* and *T. forsythia*.

Izui *et al.*^[23] studied that the curcumin inhibited the growth of *P. gingivalis*, *P. intermedia*, *Fusobacterium nucleatum*, and *T. denticola* in a dose-dependent manner. The growth of bacteria was suppressed completely at very low concentrations of curcumin. Alternatively, 100 μ g/ml of curcumin did not suppress the growth of *A. actinomycetemcomitans*, whereas at a low concentration, 20 μ g/ml of curcumin strongly inhibited *P. gingivalis*.

Shahzad *et al.*^[24] reported that the planktonic growth of periodontopathic bacteria, such as *A. actinomycetemcomitans*, *F. nucleatum*, and *P. gingivalis*, was inhibited by curcumin.

Najafi *et al.*^[25] concluded that curcumin is an effective substance in preventing the growth of *A. actinomycetemcomitans*, whose impact is reinforced when used simultaneously with photodynamic therapy and also stated that chlorhexidine had a significantly lower MIC than curcumin.

Mallikarjun and Bhat^[26] studied the ability to exert antibacterial effect of curcumin against *A. actinomycetemcomitans* at a minimum concentration of 0.2 μ g/ml.

Singh *et al.*^[27] have proved in the study the dual nature of curcumin that inhibited *P. gingivalis* lipopolysaccharide (LPS)-induced tumor necrosis factor- α and interleukin-1 β (IL) production and the inhibition of these cytokines that contributed

in reducing the impact of cytokine-mediated tissue destructive process in periodontitis.

Kim^[28] studied that curcumin had inhibited the growth of *P. gingivalis* LPS-induced cytokine expression and suppressed the production of IL-6 at both gene transcription and translation levels in *P. intermedia*.

Bakır et al.^[29] reported that curcumin acts a host modulatory agent in periodontal disease pathogenesis regarding IL-17/IL-23 axis, with a decreasing effect on alveolar bone loss and gingival expressions of IL-17 and retinoic acid receptor-related orphan receptor γ_1 .

Bhatia et al.^[30] proved the bactericidal activity of curcumin against *P. gingivalis* and *P. intermedia*.

Muglikar et al.^[31] in their pilot study evaluated the efficacy of curcumin as a therapeutic approach for the treatment of chronic periodontitis. In the clinical study, patients with chronic gingivitis had underwent scaling and root planing, followed by curcumin mouthwash (20%) for 21 days, showed significantly greater improvement of gingival inflammation, compared to the patients who had been treated only scaling and root planing.

Bhatia et al.^[30] examined that the patients with 1% curcumin gel along with scaling and root planing showed improvements in clinical parameters and reductions in the count of periodontopathic bacteria and were seen after 6 months, comparing with control sites receiving scaling and root planing only. Curcumin was used with scaling and root planing in the treatment of gingivitis and chronic periodontitis.

The antibacterial effect of curcumin and LED laser on other bacteria including *S. mutans* and *Lactobacillus acidophilus* was assessed in a study conducted by Araújo et al., in 2014.^[32] They also reported the same results indicative of a better effect in a simultaneous use of these methods. As mentioned before, the more aggressive complications of *A. actinomycetemcomitans* make this bacteria a more important target in research, which was focused in our study.

A study done by Gottumukkala et al.^[33] had showed a remarkable improvement in all the clinical and microbiological parameters on *T. forsythia*, *T. denticola*, and *P. gingivalis*, and certain strains of *Capnocytophaga* species were seen in both test and control groups on subgingival irrigation of an indigenous 1% curcumin solution in chronic periodontitis patients in a pilot randomized clinical trial when used as an alternative to scaling and root planing.

Bhandari and Shankwalker^[34] studied curcumin in the form of mouthwash and found it to be an effective anti-inflammatory agent. Since curcumin has been found to possess antimicrobial property, it has been suggested that may be used as an alternative antimicrobial agent against severe bacterial infections.

Bhat and Mandrolī^[35] studied curcumin's antibacterial activity against *Streptococcus mutans*, *Lactobacillus casei*, *Actinomyces viscosus*, *P. intermedia*, *P. gingivalis*, and *Enterococcus faecalis* and proved the use of curcumin which may be applicable in the medicament for the treatment of various endodontic diseases.

Curcumin exhibits the antimicrobial action of cefixime, cephalexin, vancomycin, and tetracycline that may be used in combination with other medicaments.^[36]

Waghmare et al.^[37] evaluated the effects of CHX and curcumin mouthwashes in the prevention of plaque formation and inflammation. The results suggested these mouthwashes as effective complementary measures alongside mechanical removal for periodontal diseases, showing a comparable effect between curcumin and CHX.

Drake et al.^[38] studied and compared to curcumin and curcumin mouthwash with chlorhexidine group, showing the antibacterial effect of curcumin (turmeric). Bacterial colonies were inoculated into tryptic soy broth-enriched cultures. The results of this study showed that the curcumin inhibited the growth of *C. gingivalis* and *P. melanogenica*.

The *in vitro* investigation of three new compounds of curcumin, namely, indium curcumin, indium diacetyl curcumin, and diacetyl curcumin, against *S. aureus*, *S. epidermis*, *E. coli*, and *P. aeruginosa* revealed that indium curcumin had a better antibacterial effect compared to curcumin itself and may be a good compound for further *in vivo* studies. However, diacetylcurcumin did not exhibit any antibacterial effect against tested bacteria.^[39]

Curcumin, which possesses both anti-bacterial and anti-inflammatory properties, can be considered as ideal for developing into medicaments with a range of possible applications in periodontal procedure. Curcumin can be used as effective alternative agent. However, further studies on large population using varied concentrations of drug may be required to improve the substantivity of the drug and also to prevent early recolonization of periodontal pathogens in chronic periodontitis cases over longer period of time. If indefinite studies on therapeutic effects of curcumin keep on increasing across the globe, it appears that curcumin truly holds a promising future in therapeutic applications including dentistry.

Conclusion

Our study has proved and upholds the use of curcumin as most natural product which is safe and can be used in dentistry.. These studies make it clear that a simple chemical structure such as that of curcumin can interact with multiple molecular targets involved in a wide variety of diseases. The affinity toward these targets could vary a great deal, from the pM to mM range. For drug development, however, the safety, efficacy, and affordability of the drug are important criteria. Curcumin meets most of these criteria. *In vitro*, *in vivo*, and human clinical studies have all established curcumin's promise and revealed its therapeutic value. More clinical studies are needed to further realize its potential. Mouthwash containing curcumin can be effectively used as an adjunct to mechanical plaque control in the prevention of plaque and gingivitis possessing anti-plaque, anti-inflammatory, and antimicrobial properties being biocompatible and well accepted by all the subjects without side effects. Substantivity of curcumin in mouthwashes is required to be studied in the future.

“With such a wide range benefits of curcumin, there is much more to say, but for now it concludes” that curcumin, an ancient spice, has proven its versatility as multipotent agent against periodontal disease. One can look forward to this herb as an adjuvant to standard therapy so as to overcome side effects.

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