COMPARATIVE EVALUATION OF ANTI-MICROBIAL EFFICACY OF CURCUMA ORAL GEL AND CHLORHEXIDINE GEL ON THE PERIODONTAL PATHOGENS: AN IN-VITRO STUDY

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ABSTRACT
Aim: To comparatively evaluate the antimicrobial efficacy of 1% Curcuma Oral Gel with 2% Chlorhexidine Gluconate gel on gram negative periodontal pathogens: Porphyromonas Gingivalis (P. Gingivalis), Prevotella Intermedia (P. Intermedia), Fusobacterium Nucleatum (F. Nucleatum) and Aggregatibacter Actinomycetemcomitans (A. Actinomycetemcomitans) in in-vitro conditions.

Materials and Method: Nutrient agar plates were inoculated by rubbing sterile cotton swabs dipped into bacterial suspensions of P. gingivalis, P. intermedia, F. nucleatum and A. actinomycetemcomitans over the entire surface of the plate. Three such sets were prepared. After inoculation, 10 mm diameter five wells were cut into the surface of each agar plate. 1% Curcuma Oral gel and 2% Chlorhexidine gel were added into wells in 4 different plates containing above mentioned 4 different bacteria and distilled water was used. Plates were incubated and the diameter of zone of inhibition was measured. The mean score of zones of inhibition were calculated and statistical analysis was done.

Results: 2% Chlorhexidine gel had edge over 1% Curcuma gel in inhibiting the growth of micro-organisms. 1% Curcuma gel was more effective against P. intermedia compared to P. gingivalis, F. nucleatum and A. actinomycetemcomitans. 2% Chlorhexidine gel was more effective against A. actinomycetemcomitans and F. nucleatum compared to P. gingivalis and P. intermedia.

Conclusion: 1% Curcuma oral gel can create a new horizon in the field of chemical agents that can be used as an adjunct to mechanical periodontal therapy and can serve as a cheaper alternative compared to 2% Chlorhexidine gel.

Keywords: Chlorhexidine gel, Periodontal Pathogens, Turmeric gel

INTRODUCTION
Periodontal disease eventually leads to tooth loss, if left untreated. It is among the most widespread oral bacterial disease.\(^1\) Although bacteria belonging to more than 630 different taxa exist in the oral cavity, only 10–15 bacterial species are recognised as potential periodontal pathogens.\(^2\) Of them, Porphyromonas gingivalis, Fusobacterium nucleatum, Prevotella intermedia and Aggregatibacter actinomycetemcomitans are recognised as the major pathogens for initiation and progression of destruction of tooth supporting structures. The levels of P. gingivalis, P. intermedia and other anaerobic bacteria are seen to increase in adult onset periodontitis. While P. gingivalis and A. actinomycetemcomitans are strongly associated with localised aggressive periodontitis, P. intermedia is predominantly associated with the development of necrotising ulcerative gingivitis.\(^3\) Longitudinal and retrospective studies have demonstrated an increased risk of periodontal breakdown in A. actinomycetemcomitans and P. gingivalis positive sites and better post-treatment results in their absence.\(^4\)

India has a rich history of using plants for medicinal purposes. Turmeric (haldi), a rhizome of Curcuma longa, is a common antiseptic used in traditional system of Indian medicine. It may be a more acceptable and viable option for the common man. It has proven properties like anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, immunostimulant, antisptic, antimutagenic, and it also accelerates wound healing.\(^5\) Curcumin, the main yellow bioactive component of turmeric, has been shown to have a wide spectrum of biological actions.\(^6\) Literature reports have shown that curcumin has anti-inflammatory and antibacterial activities, suggesting its potential to be used as a subgingival agent.\(^7\) Safety evaluation studies have indicated that both turmeric and curcumin are well tolerated at a very high dose without any toxic effects.\(^7\) To the best of our knowledge, there are currently no studies assessing the efficacy of turmeric gel with the various periodontal pathogens, using the disc diffusion method. Hence, the present study will comprehensively report the antimicrobial potential of turmeric gel on these key periodontal pathogens. It will also assess and compare the in vitro efficacy of turmeric gel with the gold standard 2% chlorhexidine gel.
MATERIALS AND METHODS

The study was designed as an in vitro study. Before conducting the study, necessary ethical approval was obtained from the Institutional Ethics Committee. Other related approvals from the concerned authorities were obtained for the necessary microbial analysis. Materials used in the study were 1% turmeric gel, 2% chlorhexidine gel and distilled water. Microorganisms included were *P. gingivalis*, *P. intermedia*, *F. nucleatum* and *A. actinomycetemcomitans* strains. Test materials included blood agar plates, Petridishes and a digital Vernier caliper. A total of 60 samples were taken. Samples were divided into 3 reagent groups, as mentioned above × 4 strains of microorganisms × 3 repetitions.

**Disc diffusion method**

The antibacterial activity of 1% turmeric gel and 2% chlorhexidine gel 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. Alternatively, the suspension was standardised with a photometric device. Within 15 min of adjusting the inoculums to a McFarland 0.5 turbidity standard, a sterile cotton swab was dipped 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 min, but no longer than 15 min before making wells. A hollow tube of 5 mm diameter was heated and pressed above the inoculated agar plates and was removed immediately, making a well in the plate. Likewise, four wells of 5 mm diameters each were made on each plate. 1% turmeric gel, 2% chlorhexidine gluconate gel and distilled water were added in the wells assigning each plate for each of the solution. Within 15 min of compound application, plates were shifted to an anaerobic jar, which was kept in an incubator 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 caliper. The mean score of zones of inhibition was calculated for each solution, respectively.

**Table 1**: Mean zone of inhibition comparing Curcuma gel and chlorhexidine gel for Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Aggregatibacter actinomycetemcomitans (Aa), Fusobacterium nucleatum (Fn).

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Curcuma oral gel (1%)</th>
<th>D/W</th>
<th>Chlorhexidine gluconate gel (2%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pg</td>
<td>12mm</td>
<td>R</td>
<td>16mm</td>
</tr>
<tr>
<td>Pi</td>
<td>13mm</td>
<td>R</td>
<td>16mm</td>
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<tr>
<td>Aa</td>
<td>12mm</td>
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<tr>
<td>Fn</td>
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RESULTS
2% Chlorhexidine gluconate gel showed the greatest zone of inhibition against all periodontal pathogens. 1% turmeric gel showed 13 mm zone of inhibition against P. intermedia whereas 12 mm zone of inhibition was seen against P. gingivalis, A. actinomycetemcomitans and F. nucleatum. (Table-1)

DISCUSSION
The consensus report of the American Academy of Periodontology proved that the micro-organisms responsible for periodontitis are A. actinomycetemcomitans, P. gingivalis, P. intermedia, F. nucleatum. According to specific plaque hypothesis, by eliminating above mentioned species, periodontal disease would regress. Thus periodontal health of the individual can be established with the help of the agents like turmeric gel along with the use of mechanical plaque control therapy.

The combination of microorganisms and inflammatory response are the cause of many diseases, including periodontitis, for which compounds having a dual anti-inflammatory and antimicrobial activity can be desirable therapeutic agents. The results of this study corroborate with the findings of study by Mandrola et al, which was done to evaluate the ability of curcumin to exert antibacterial activity against common endodontic bacteria like Streptococcus mutans, Actinomyces viscosus, Lactobacillus casei, P. gingivalis, Prevotella intermedia and Enterococcus faecalis. It was, therefore, suggested that curcumin has the ability to kill several Gram-positive and Gram-negative bacteria.

A study by Rai et al (2008) suggested that curcumin may inhibit bacterial cell proliferation by inhibiting the assembly dynamics of FtsZ (a bacterial protofilament), which polymerizes to form a Z-ring at the midcell that orchestrates bacterial cell division. The assembly and stability of FtsZ protofilaments have been shown to play critical roles in bacterial cytokinesis. Thus, FtsZ may be considered as an important antibacterial drug target. Another reason which might be suggested for the antibacterial potential of curcumin is that curcumin is a polyphenolic compound, also having substantial antibiofilm activity. It inhibits the production of biofilm and disperses the biofilm made by many microorganisms.

Owing to the plaque biofilm acting as the main etiological factor in the periodontal diseases, curcumin's antibiofilm activity would also account for its antibacterial activity against periodontal pathogens. Apart from the antibacterial property, curcumin is a molecule known to demonstrate antifungal activity and antiviral activity as well. Further, curcumin has long been touted as a powerful anti-inflammatory and immunomodulatory agent.

Curcumin has been shown to regulate numerous transcription factors, cytokines, protein kinases, adhesion molecules, redox status and enzymes that have been linked to inflammation. The anti-inflammatory activity of curcumin was first reported in 1971. It reduces inflammation by effectively inhibiting transcriptional and translational expression of proinflammatory cytokines like Interleukin – 6 (IL – 6) and Tumour Necrosis Factor – alpha (TNF – α). Curcumin also demonstrated dose dependent attenuation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kb) activation in the gingival tissue of rats suffering from experimental periodontal disease. It, further, reduced the inflammatory infiltrate, increased collagen content, and increased fibroblastic cell numbers in gingival tissues.

Despite extensive research and development, poor oral bioavailability of curcumin remains one of the major concerns for its use as a therapeutic agent. This poor oral bioavailability of curcumin can be attributed to its poor absorption, high rate of metabolism in the intestines and rapid systemic elimination from the body. Thus, attempts have been made to improve curcumin’s bioavailability by use of adjuvants that can block the metabolic pathway of curcumin. This has been achieved through encapsulation in liposomes, biodegradable microspheres, cycloexdrin, and hydrogels. Also, various controlled delivery forms, such as polymeric micro/ nanospheres, liposomes, micelles, parenteral emulsion, and prodrugs have been investigated to increase its solubility.

Maintenance or improvement of oral health by use of natural products that also form a part of habitual diet seems to be a suitable way to preserve oral health. Patients will tend to be compliant as they are not burdened with added cost or time. However, this cannot be confirmed without appropriate studies. Additionally, proper utilization of available natural resources to reduce the ever increasing prevalence of oral diseases in rural parts of developing countries can help to diminish the dangers of a poor dentist-population ratio.

Active ingredients from such potent natural resources should also be evaluated for their anti-inflammatory and immune-modulatory properties. It is recommended that the readily accessible natural products may be integrated with presently available synthetic materials that are used to maintain the oral hygiene.

However, any natural product should be assessed for its safety and clinical application should not be done without sufficient scientific evidence. The mechanism of action of a particular natural product needs to be studied at a molecular level in order to assure the accuracy of application and consequently, the outcomes. Although none of the nutraceuticals used in this study are known...
to have side effects, oral retention and duration of action of these phytoplants is an important factor that needs further exploration. Thus, from this study one could say a wide plethora of new dimension in nonsurgical periodontal therapy can open up, if turmeric gel can be used as adjunct to nonsurgical periodontal therapy along with chlorhexidine digluconate.

CONCLUSION

The various in-vitro and clinical studies have shown that curcuma oral gel has the potential to inhibit various micro-organisms which cause periodontitis. Curcuma oral gel does not have any adverse reactions reported till date. Thus, its use should be increased for the patients with hypersensitivity towards chlorhexidine, which would give the periodontist another adjunctive treatment option along with scaling and root planing. 2% Chlorhexidine gel is better than 1% Curcuma gel but looking at the cost factor curcuma gel can be a cheaper alternative to chlorhexidine gel. However, more microbial as well as clinical studies should be undertaken to establish the results of the present study. Clinical trial should be encouraged so as to reap the benefits of natural products like turmeric, especially in the field of periodontal local drug delivery and non surgical periodontal therapy in the management of periodontitis.

REFERENCES