



## Evaluation of antimicrobial effectiveness of a novel turmeric-chitosan based gel – An in-vitro study

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### ABSTRACT

**Background:** Chitosan is a highly biocompatible compound with enhanced substantivity. It possesses antifungal antibacterial and properties. However, sufficient data does not exist on the response of chitosan to oral pathogens. Also, the use of chitosan in combination with other naturally occurring substances like turmeric can be studied in order to obtain an organic solution to lesser infections following oral procedures.

**Materials and methods:** A chitosan-turmeric gel was prepared in the lab. Following this, a Kirby-Bauer disk diffusion test was done to assess the sensitivity of the various oral pathogens to the novel gel that was compared with the positive control, for which commercially available antibiotics were used. The zone of inhibition was measured for three different dilutions of the gel, 25µL, 50µL, and 100µL.

**Results:** It was found that the turmeric chitosan gel in the dilution of 100 µL was found to have the largest value for the zone of inhibition against *E. Faecalis*, *S. aureus*, *S. mutans* and *C. Albicans* amongst the three groups of dilutions. The zone of inhibition was approximately 50% that of the control group (antibiotic) for the 100 µL dilution. The zone of inhibition for the 100 µL dilution was more than that of the control against *C. Albicans*.

**Conclusion:** The turmeric chitosan gel is beneficial in preventing microbial infections. However, in vivo studies need to be conducted to confirm the findings.

**Keywords:** antimicrobial activity, green synthesis, chitosan, turmeric

## INTRODUCTION

Chitosan is a natural polysaccharide made up of -(14)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine that are randomly distributed) (acetylated unit). It's manufactured by treating shrimp and other crustacean chitin shells with an alkaline chemical like sodium hydroxide. In medicine, it is useful in wound dressings as hemostatic agent, antibacterial agent, and as a drug delivery agent through skin. Recently, newer products containing chitosan in a gel formulation are being developed focussing on the hemostatic potential of chitosan post-surgical procedures(Azargoon et al., 2011; Kale, 2012; Kumar et al., 2016)(J et al., 2018). A chitosan brush has been created as a potential method for removing plaque in a professional setting. Evidence indicates that chitosan brushing can decrease inflammation and bleeding around dental implants, while also stabilizing bone levels. (Zeza, Wohlfahrt and Pilloni, 2017). Chitosan membranes have been evaluated as a potential option for guided bone regeneration.(Ali and Ahmed, 2018)

The most commonly undertaken oral surgical procedures include third molar extractions, implant placement, biopsies, resection of a tumour or any other existent pathology. Such procedures are commonly identified as unpleasant by the patient. The most common and relevant postoperative problems associated with intraoral surgeries include pain, inflammation and defective wound healing process.(Friedman, 2007)(Sridharan et al., 2019) A prerequisite for undisturbed oral wound healing is infection control and is essential for the transition of the inflammatory to the proliferative phase (Larjava, 2013). Peri-implant soft tissue healing has also become an important clinical outcome following implant placement since it governs the esthetic outcome of the patient. Peri-implant soft tissue healing follows the same principles as gingival healing around a tooth post-surgical operations(Villar et al., 2011). In order to prevent side effects of systemically administered drugs, locally administered drugs should be considered. Several synthetic chemicals such as amoxicillin, metronidazole, tetracycline, clindamycin, ofloxacin etc. have been evaluated as an alternative to the systemically administered drugs.(Ramesh et al., 2018)(Ramadurai et al., 2019) However, these can lead to bacterial resistance, opportunistic infections and allergic reactions, specially in patients in a compromised

immune function. Instead, natural substances can be used as medicinal agents.(Siddique et al., 2019) Around 81% of the population in developing countries relies on natural medicines for their medical health problems. (Palombo, 2011).

Chitosan has been praised for its antibacterial and antifungal properties, as well as its biocompatibility. It has been previously documented as a drug delivery mechanism and a means of stopping bleeding.(Teja, Ramesh and Priya, 2018) A synergistic effect has also been observed by combining chitosan with existing chemical products like chlorhexidine in promoting antimicrobial activity at the surgical site (Decker et al., 2005)(Ezhilarasan, Apoorva and Ashok Vardhan, 2019). Various other synthetic drugs have been established to date as antimicrobial, anti-inflammatory and analgesic agents.(Vijayashree Priyadharsini, 2019) Amongst these, chlorhexidine digluconate has been established as the safest and most efficient option. Hence it has gained popularity as an oral antiseptic agent over the recent years to reduce postoperative intraoral pain and infection (Balejo et al., 2017). However, chlorhexidine has side effects like staining teeth and prostheses, epithelial desquamation.(Mathew et al., 2020)

Over the recent years, polymers that are both biodegradable and mucoadhesive, including chitosan, alginate, and gelatin, have become increasingly popular as a means of delivering antimicrobial agents in slow-release devices.(Duraisamy et al., 2019) Nevertheless, whether or not such gel formulations are actually beneficial in reducing the risk of microbial infection at the site hasn't been established yet. Furthermore, such gels' potential advantages and disadvantages compared to other already existent treatment modalities have not been established in the literature to date. Therefore, the objective of the present study was to assess in vitro the antimicrobial effectiveness of a novel chitosan turmeric gel against oral pathogens.

## MATERIALS AND METHODS

### *Gel Preparation*

For the preparation of the chitosan-turmeric gel, 0.5 g chitosan was dispensed in 0.5 mL of acetic acid and 24.5 mL of distilled water. This was stirred until a homogenous mix was obtained. 2.5 g of turmeric was added, and the solution

obtained was put on a stirrer. A few drops of glacial acetic acid were added to the mix. After this, 2.5 g of carbopol was added gradually to the

mix and stirred. The prepared gel was then kept in a sonicator for a uniform mix (Figure 2).

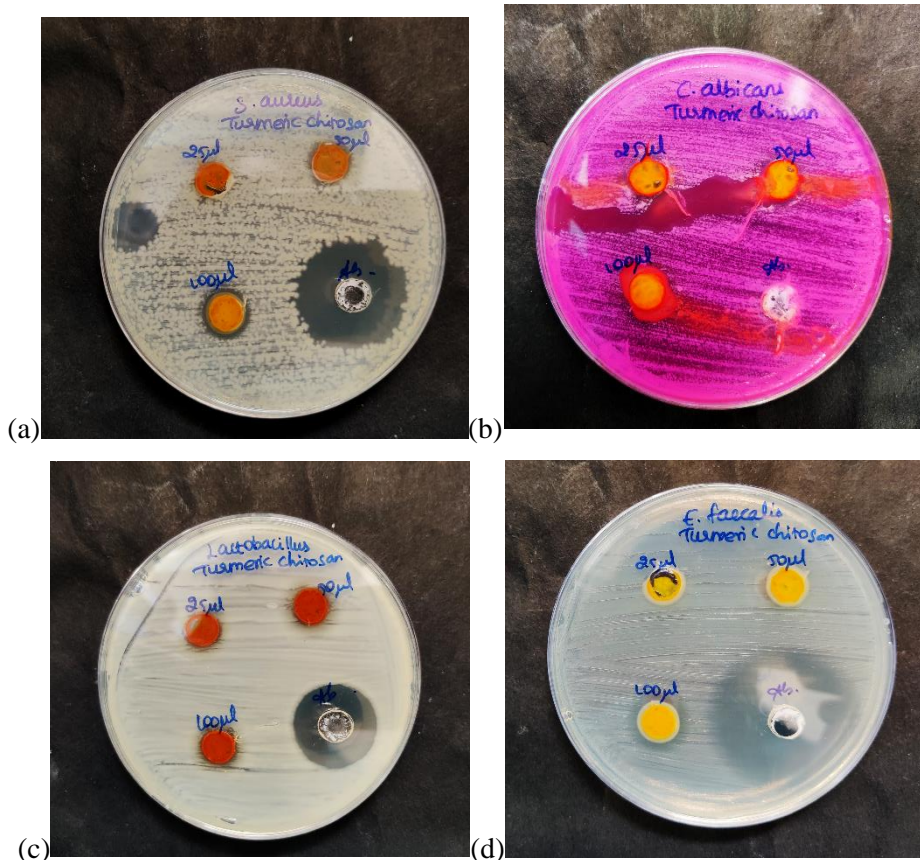


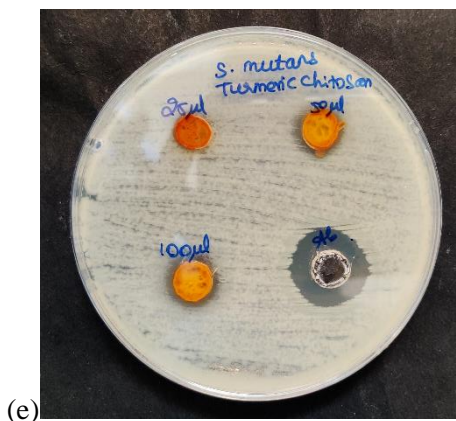
FIGURE 2: Steps in the preparation of the turmeric-chitosan gel

### Evaluation of Antimicrobial Activity

For the evaluation of antimicrobial activity, the Kirby-Bauer Disk Diffusion method was used. For this, agar plates were prepared for 5 different microorganism species commonly found in the oral environment during infection. The included species for the study were - *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus*

*mutans*, *Lactobacillus* species, and *Candida albicans*. Different concentrations (25  $\mu$ L, 50 $\mu$ L, 100 $\mu$ L) of the prepared Turmeric Chitosan gel was added to wells in agar plates. The agar plates were then incubated at 37°C for 24 hours. (Figure 3). After 24 hours, the zone of inhibition for each concentration was measured using a Vernier Caliper.





**FIGURE 3:** Kirby-Bauer disk diffusion susceptibility test for different microorganisms. (a) *Staphylococcus aureus*; (b) *Candida albicans*; (c) *Lactobacillus* species; (d) *Enterococcus faecalis*; (e) *Streptococcus mutans*

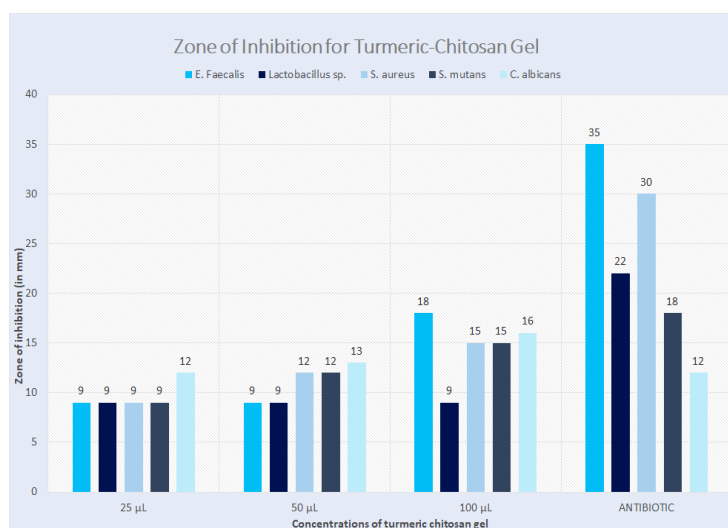
**RESULTS**

The zone of inhibition obtained by the various concentrations of the gel and the control (in mm)

against different oral pathogens is presented in Table 1. The findings have been represented in the form of a clustered bar graph in Figure 1.

**TABLE 1:** Zone of inhibition obtained by the various concentrations of the gel and the control (in mm) against different oral pathogens

	E. Faecalis	Lactobacillus sp.	S. aureus	S. mutans	C. albicans
25 µL	9	9	9	9	12
50 µL	9	9	12	12	13
100 µL	18	9	15	15	16
Antibiotic	35	22	30	18	12



**DISCUSSION**

The antimicrobial property of the novel chitosan turmeric gel was evaluated by the Kirby-Bauer Disk Diffusion method (Andrews, 2002). The turmeric chitosan gel in the dilution of 100 µL

was found to have the highest value for the zone of inhibition against *E. Faecalis*, *S. aureus*, *S. mutans* and *C. Albicans*, three groups of dilutions. It was seen that the zone of inhibition for the 100 µL dilution was approximately 50%

of that of the control antibiotic against *Staphylococcus aureus* and *Streptococcus mutans*. The zone of inhibition for the 100  $\mu$ L dilution was more than that of the control antibiotic against *C. Albicans*.

The turmeric chitosan gel had promising values for the zone of inhibition in the concentration of 100  $\mu$ L against *E. Faecalis*, *S. aureus*, *S. mutans* and *C. Albicans*. The zone of inhibition against *S. mutans* was 15 mm for the 100 $\mu$ L dilution of the gel, which was nearly similar to the zone of inhibition obtained by the antibiotic control group, which was 18 mm. The zone of inhibition for the 25  $\mu$ L and the 50  $\mu$ L dilutions was comparatively less compared to the control and hence can be considered ineffective. To date, there is no available literature on the antimicrobial effectiveness of a combination of turmeric and chitosan. However, a study has been done in the past assessing the antimicrobial activity of a similar chitosan-based gel with chlorhexidine as a constituent. The assessment was done by calculating the colony-forming units on the suture threads isolated from the area, in contrast with the disk diffusion method used in the current study. It was found that the antimicrobial activity was not statistically significant when compared to the control, for which a placebo was used (Rodríguez Zorrilla et al., 2020).

The zone of inhibition for the turmeric chitosan gel against *E. faecalis* and *S. aureus* was 18 mm and 15 mm in 100  $\mu$ L dilution compared to the control group values of 35 mm and 30 mm, respectively. Hence the antimicrobial activity of the gel against these pathogens can be considered as approximately 50% of the positive control. Previously, attempts have been made to understand the mechanism of action of chitosan as an antimicrobial agent. The mechanism of action that is most commonly proposed involves binding of the substance to the negatively charged bacterial cell wall, which disrupts the cell and alters the permeability of its membrane. Following this, the substance gets attached to the bacteria's DNA, preventing replication and ultimately leading to the death of the cell.(Wang, Hu and Shao, 2017). Thus, the gel can be considered to promote antimicrobial activity against these pathogens in 100  $\mu$ L dilution, though it may not be as effective as the control. The dilutions of 25  $\mu$ L and 50  $\mu$ L show a remarkably less inhibition zone than the control

and hence should not be considered for future use.

The response of the chitosan turmeric gel against *C. Albicans* is the most promising. The zone of inhibition obtained from the gel in the concentration of 100  $\mu$ L dilution was 16 mm, which was greater than that obtained by the positive control used for the study (12mm). This finding agrees with a previous study that concluded that chitosan has a significant antifungal effect against *C. Albicans*(Shih et al., 2019). The mechanism of action of chitosan as an antifungal agent has been explained in the study by Shih et al., who found that treatment of *C. Albicans* cells by chitosan can lower the levels of  $\beta$ -glucan and chitin and alter the cell membrane and cell wall ultrastructure by inhibiting the expression of SAGA complex gene. Hence the gel can be considered to have high antifungal activity and prove to be very effective in promoting good wound healing after oral surgical procedures like extractions and implant surgeries and promoting recovery from conditions like denture stomatitis and other candidal infections. However, studies haven't been done to compare the antifungal effect of chitosan when combined with a natural constituent like turmeric, which can provide a synergistic effect and other benefits.

A few limitations can be considered for the current study. The study included single readings for a particular dilution of gel against a specific microorganism. The findings would have been more accurate if a mean value was calculated from multiple readings. Also, the study was conducted in a laboratory setup. The findings of the study might vary when conducted in the oral environment. Further studies can be conducted to assess the Minimum Inhibitory Concentration (MIC) of the novel chitosan turmeric gel prepared in the study to get the concentration at which the gel will be the most effective. Also, confirmatory in vivo tests can be performed by calculating the CFUs (colony forming units) of the various species of microorganisms in response to the novel gel.

## CONCLUSION

It can be concluded that the turmeric-chitosan gel is beneficial in preventing microbial infections. Encouragement of its use is based on a scientific understanding of its advantages and correct

usage. Affordability and the easy availability of the gel constituents in certain regions could be especially helpful for developing countries with limited access to oral healthcare and financial resources.

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