

**STUDY OF THE ANTIBIOFILM AND ANTIPLANKTONIC EFFECT OF A
COMMERCIALY AVAILABLE ORAL HYGIENE PRODUCT**

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ABSTRACT

Oral medicine is an area of dentistry which is constantly changing. Colonization of bacteria causes various diseases of oral cavity such as plaque and gingivitis. The recent scientific advances and research in oral medicine focused on the improvement of oral health by using certain chemical therapy in the form of various formulations of mouthwashes as an oral hygienic product. The rising awareness among customers regarding dental health has resulted in mouthwashes gaining importance, due to the fact that they include diverse types of antimicrobial agents to complement the results of mechanical oral hygiene measures. The purpose of the study was to study the antibiofilm and antiplanktonic effect of a commercially available oral hygienic product Listerine on the suspended and sessile state of *Streptococcus mutans*. The present work was designed to screen the antibacterial action of a mouthwash and to carry out an exposure time analysis to check its effect on the growth of planktonic form and biofilm development. The study revealed an excellent antibacterial action of the product and on comparing the antibiofilm and antiplanktonic effect, the product was found to have a good antiplanktonic effect.

KEYWORDS: *Streptococcus mutans*, Listerine, Mouthwash, Antibiofilm, Antiplanktonic.

INTRODUCTION

Oral flora are the main cause of prevalent oral diseases. Caries and inflammatory periodontal disease are the most prevalent oral diseases, and both result from the activity of dental bacterial plaque. Plaque is a complex biofilm that contains various microorganisms and forms mainly on teeth and particularly between them, along the gingival margin, and in fissures and pits. This biofilm adheres by a variety of mechanisms. If plaque is not removed regularly, the flora evolves and plaque may calcify, forming calculus. For this reason, plaque control plays a significant role in the prevention of gingival and periodontal problems. Both mechanical aids of tooth cleaning and local chemotherapeutics are used for this purpose.^[1] It has been recognized that both regular supportive periodontal therapy and the domiciliary oral hygiene, related to supragingival plaque control through a host modulation of periodontal bacteria, are essential for obtaining a good clinical outcome.^[2] Biofilm formation, which is a bacterial aggregation between bacteria, may play an important role in the accumulation of oral bacteria on teeth. These highly selective interactions may contribute to the organization of microflora at specific sites and can be difficult to control.^[3] Improvement of oral health by controlling biofilm formation can influence the life quality of oral

hygiene, so development of new preventive products which are safe, effective and economical is necessary. Mouthwashes (mouthrinses) are solutions or liquids used to rinse the mouth for a number of purposes: (a) to remove or destroy bacteria (b) to act as an astringent (c) to deodorise and (d) to have a therapeutic effect by relieving infection or preventing dental caries. Constituents of mouthwashes include water (chief constituent); ethanol, dyes, surface active agents, zinc chloride /acetate, aluminium potassium sulphate (astringent); and phenolic compounds, quaternary ammonium compounds and essential oils such as oil of peppermint (as antibacterial agents) among others. Mouthwashes also provide a safe, effective chemical means of reducing or eliminating plaque accumulation.^[3,4] In this context, the use of a chemical antiseptic i.e. a mouthwash is studied to check its effect in inhibiting the plaque biofilm formation. This could be fundamental to support the mechanical therapy in preventing dental caries and plaque formation.^[5] In particular, their antimicrobial effect has been tested by calculating the broth dilution method i.e. Minimum Inhibitory dilutions as well as by agar cup method and exposure time analysis. The results will allow to estimate the effect of mouthwash, Listerine on planktonic growth and on biofilm development of *Streptococcus*

mutans.

MATERIALS AND METHODS

Subject- A healthy volunteer showing the presence of dental caries on oral examination participated in the experiment. The dental swab was taken before the routine oral hygienic procedures.

Test culture- The test culture was obtained after performing the isolation of dental swab from the oral cavity of the volunteer.

Mouthwash- Listerine (original) manufactured and distributed by Johnson & Johnson Private Limited was used as the mouthwash sample.

Culture media- Mueller Hinton agar and Mueller Hinton broth obtained from Himedia Laboratories, India was primarily used as the culture media.

Isolation and identification of the dental flora

Isolation of the organisms from the dental swab was carried out on st Mueller Hinton agar plates and incubated for 24 hrs. at 37°C. An isolated colony was selected, its microscopic, macroscopic and biochemical characteristics were studied and the isolate was identified using The Bergey's Manual. A pure culture of the isolate was maintained on st. Mueller Hinton slants i.e. they were sub cultured and stored at 4°C for further usage.

Preparation of inoculum

- For studying the antibiofilm effect – A loop full of the pure culture was inoculated into 10ml of st. Mueller Hinton broth in 250ml flask. It was incubated in a static position for 24hrs. After incubation the suspension was vortexed to dislodge the culture. This suspension was adjusted to an optical density of 0.1 units at 530 nm and used for further assays.
- For studying the antiplanktonic effect – A loop full of culture was inoculated in 10ml st. Mueller Hinton broth. It was kept in the rotary shaker for 24hrs. The suspension was then adjusted to an optical density of 0.1 units at 530 nm and used for further assays.

Preparation of mouthwash dilutions

Different dilutions of Listerine such as 1:2, 1:10, 1:100, 1:1000 and 1:10000 were prepared using st. distilled water as the diluent. These were used for the studying the antimicrobial activity.

Assay of antimicrobial activity

Study of the effect of different concentration of Listerine on the growth of *S. mutans* in the biofilm state and planktonic state and determination of Minimum Inhibitory Dilution

0.1 ml of the inoculum prepared was added to 0.1ml of undiluted, 1:2, 1:10, 1:100, 1:1000 and 1:10000 dilutions of Listerine and incubated for 5 min. Then, 0.1 ml aliquot

from each of the incubated mixture was added to 5 ml of st. Mueller Hinton broth and incubated at 37°C/24hrs. The tubes were observed for growth and their turbidity recorded spectrophotometrically to study the effect of the concentration of Listerine on the growth of the *S. mutans* isolate in the biofilm state and planktonic state.

Determination of antimicrobial activity by Agar cup method^[6]

St. Mueller Hinton agar plates inoculated with 0.1 ml of culture suspension were prepared. Using a st. cork borer of 10mm external diameter wells were punched into the plate to which 100µl ml of different dilutions of the mouthwash were added to the wells. The plate were kept for prediffusion at 4°C/30min and further incubated at 37°C/24hrs and the zone of inhibition was recorded.

Exposure Time Analysis

The assay was used to determine the time required for the mouthwash to achieve the bactericidal effect at its minimum inhibitory dilution. 0.1 ml of culture suspension was added to 2ml of the mouthwash at its its minimum inhibitory dilution and incubated for different time intervals (1, 2, 3, 4 and 5 min). 0.1 ml aliquot from each was added to 5 ml of st. Mueller Hinton broth and incubated at 37°C/24hrs. The tubes were then observed for growth and their turbidity recorded at 530 nm to determine the bacterial viability in relation with five different exposure times.

RESULTS AND DISCUSSIONS

Isolation and identification of the dental flora

Different organisms were isolated from the dental swab of the volunteer showing the presence of dental caries. A single colony was selected and on studying the microscopic, macroscopic and biochemical characteristics the isolate was identified as *Streptococcus mutans*.

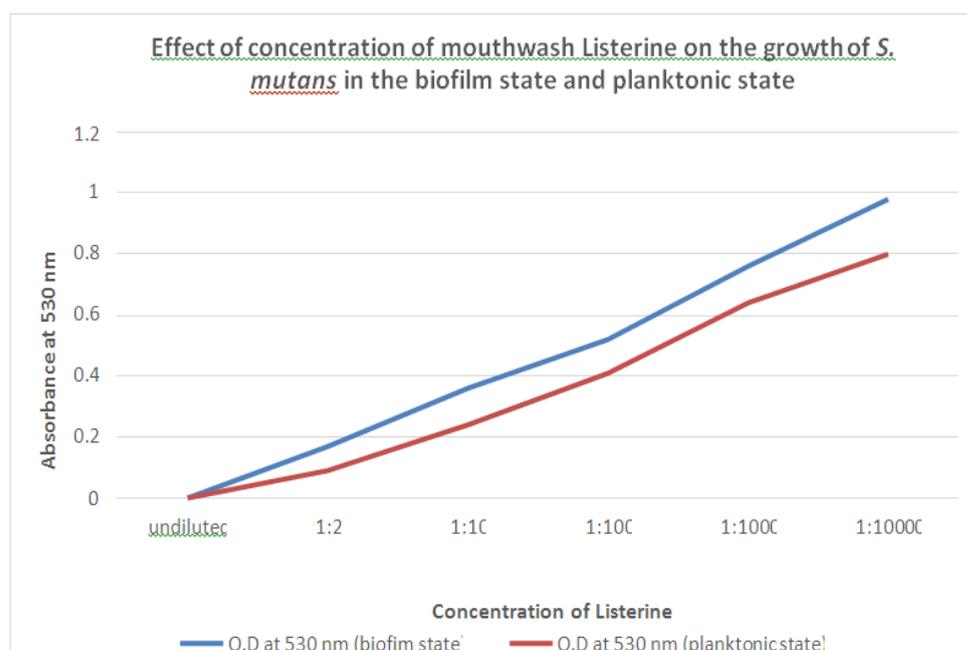
Determination of antimicrobial activity

Study of the effect of concentration of mouthwash Listerine on the growth of *S. mutans* in the biofilm state and planktonic state and determination of Minimum Inhibitory Dilution

The mouthwash, Listerine in an undiluted form was found to be inhibitory to the isolate of *S. mutans*. The dilutions 1:2, 1:10, 1:100, 1:1000, and 1:10000 were ineffective in inhibiting the culture. The effectiveness of the mouthwash Listerine in inhibiting the culture was determined visually as well as by recording the turbidity spectrophotometrically at 530 nm. The turbidity was found to decrease with decrease in the dilutions of the mouthwash and no turbidity was recorded when the mouthwash was used in the undiluted form. As seen in Table 1 and as represented in Graph 1, the optical density was found to be increasing with increase in dilutions indicating that the bacterial viability decreased as the concentration of the mouthwash increased.

Table 1: Effect of concentration of mouthwash Listerine on the growth of *S. mutans* in the biofilm state and planktonic state.

Concentration of mouthwash (Listerine)	Absorbance at 530nm (biofilm state)	Absorbance at 530nm (planktonic state)
Undiluted	0.00	0.00
1:2	0.17	0.09
1:10	0.36	0.24
1:100	0.52	0.41
1:1000	0.76	0.64
1:10000	0.98	0.80

**Graph 1: Effect of concentration of mouthwash Listerine on the growth of *S. mutans* in the biofilm state and planktonic state.****Determination of antimicrobial activity by Agar cup method**

The sensitivity of the isolate of *S. mutans* to the mouthwash was determined by performing the Agar cup method. After incubation the zones of inhibition were recorded the Table 2. The isolate showed a maximum zone of inhibition to the mouthwash in its undiluted form and no zone of inhibition was exhibited when the mouthwash was used in dilutions of 1:10, 1:100, 1:1000 and 1:10000. Thus the mouthwash in the undiluted form was found to be most effective against the isolate of *S. mutans* (Fig. 1).

Table 2: Zones of inhibition of *S. mutans* by Agar cup method.

Concentration of Listerine	Zone of inhibition (mm) of <i>S. mutans</i>
Undiluted	18
1:2	13
1:10	No inhibition
1:100	No inhibition
1:1000	No inhibition
1:10000	No inhibition

**Figure 1: Antimicrobial activity of mouthwash Listerine against *S. mutans* by Agar cup method. Exposure time analysis**

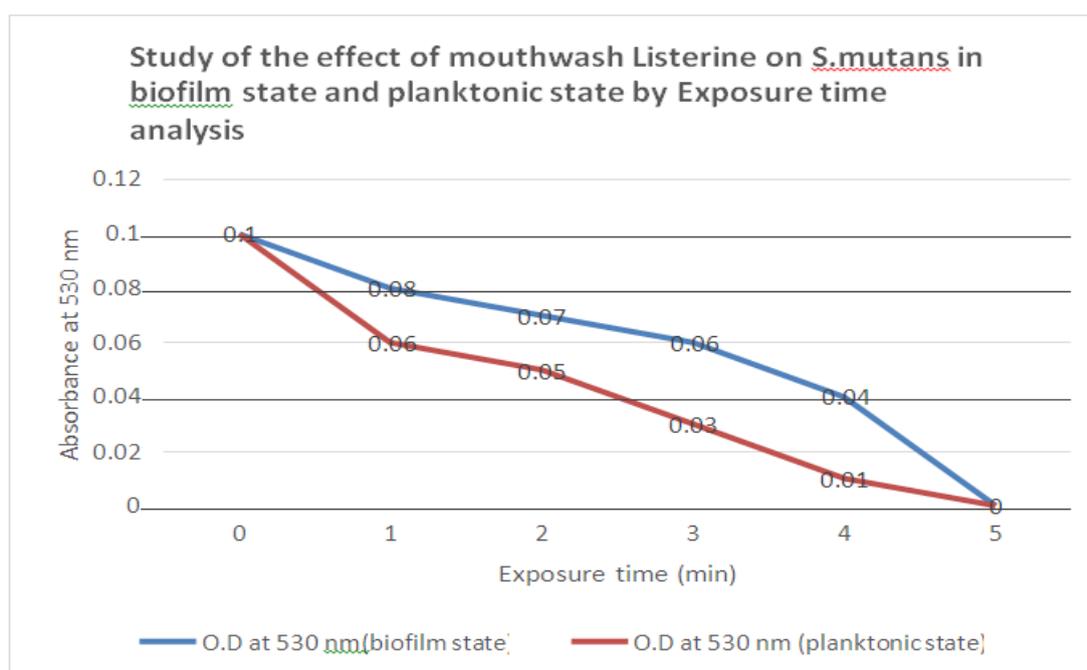
The exposure time analysis was performed to study the viability of the isolate of *S. mutans* when exposed to the undiluted form of Listerine for different time intervals. The effectiveness of the undiluted form of Listerine was

checked visually and spectrophotometrically at 530 nm. The values for the absorbance are tabulated in Table 3. It was found that as the time exposure increases the viability of bacteria decreases as depicted in Graph 2. Listerine was found to inhibit bacterial viability after 5 minutes. The state of the culture was found to influence

the effectiveness of the mouthwash, Listerine. It was found that the mouthwash showed a higher antiplanktonic effect as compared to its effect on the culture in the biofilm form. Thus indicating that the biofilm form is more resistant to the mouthwash, Listerine as represented in the Graph 2.

Table 3: Study of the effect of mouthwash Listerine on *S.mutans* in biofilm state and planktonic state by Exposure time analysis.

Time exposure (min)	Absorbance at 530 nm (biofilm state)	Absorbance at 530 nm (planktonic state)
0	0.1	0.1
1	0.08	0.06
2	0.07	0.05
3	0.06	0.03
4	0.04	0.01
5	0.00	0.00



Graph 2:- Study of the effect of mouthwash Listerine on *S.mutans* in biofilm state and planktonic state by Exposure time analysis.

CONCLUSION

From the study we can conclude that the mouthwash, Listerine showed excellent antimicrobial activity against the isolate of *S.mutans* isolated from the volunteer having dental caries. Following the completion of the three different techniques to assess the antimicrobial potential of the mouthwash, Listerine, it was possible to highlight how bacterial viability is always related to the dilution factor. Listerine showed excellent antimicrobial activity against the isolate of *S.mutans* in the planktonic state than against the organism in the biofilm form. More importantly, the test was conducted in vitro, so it cannot be assumed that the results of antimicrobial efficacy could be proportional or transferable to the oral cavity and translated into clinical effectiveness. However further studies should be conducted in order to confirm

the activity of Listerine against the biofilm forms of the isolate. On the basis of these results, it seems that an alcoholic mouthwash could be a good choice in preventing plaque formation after periodontal therapies.

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