

# Comparative evaluation of antibiofilm formation activity of *Plectranthus amboinicus* extract against *Streptococcus mutans*

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## ABSTRACT

**Aim and Introduction:** Biofilms are multimicrobial communities enclosed in self-synthesized polymeric matrices attached to biotic or abiotic surfaces. The biofilm is used to describe the various communities of microorganism attached to a surface. These are made up of microbial substances. Biofilms are highly competitive types, where some exhibit as antibiofilm. These show the characteristic features such as bacterial growth inhibition and exclusion. The present study leads to the synthesis of *Plectranthus amboinicus* extract and studying the antibiofilm formation against *Streptococcus mutans*. **Materials and Methods:** The organism *S. mutans* was isolated from saliva sample using special media [Mutans-Sanguis agar] and maintained in Tryptose soya agar at 4°C in the Department of Microbiology, Saveetha Dental College and Hospitals. Evaluating the microtiter plate with different concentrations of *Plectranthus* extract against *S. mutans* and studying the antibiofilm formation. **Results:** *P. amboinicus* extract shows significant of antibiofilm against *S. mutans*. **Conclusion:** *P. amboinicus* extract was found to be 79.43% effective than *S. mutans*. Thus, by increasing the concentration of *P. amboinicus* extract in the microtiter plate, the effectiveness of *Plectranthus* extract against *S. mutans* can be assessed in future.

**KEY WORDS:** Extract, Microtiter and antibiofilm, *Plectranthus amboinicus*, *Streptococcus mutans*

## INTRODUCTION

A biofilm is a thick layer which has aggregated to form a colony. It is from prokaryotic organisms. It is maintained through cell-to-cell communication. Biofilms are highly competitive types, where some exhibit as antibiofilm.<sup>[1]</sup> The biofilm is used to describe the various communities of microorganism attached to a surface. These are made up of microbial substances. Biofilm forms fast in flow systems where a regular nutrient supply is provided to the bacteria.<sup>[2]</sup> The biofilm allows the microorganism to undergo a wide range of physiological and morphological adaptations in response to which microorganisms must adapt to survive.<sup>[3]</sup> It is composed of bacterial cells (15–20%) and distributed in a matrix form or glycocalyx (75–80%).<sup>[4]</sup> Although the exopolymer matrix of biofilm is not significant,

the barrier in itself has the property of retarding the diffusion.<sup>[5]</sup>

*Streptococcus mutans* is a facultatively anaerobic, Gram-positive bacteria. It is commonly found in human oral cavity.<sup>[6]</sup> *S. mutans* colonize the dental surface. It causes damage to the hard tooth structure in the presence of fermentable carbohydrates, for example, sucrose and fructose.<sup>[7]</sup> *S. mutans* gives its name to a group of seven closely related species collectively referred to as the mutans streptococci. The primary habitats for *S. mutans* are mouth, pharynx, and intestine.<sup>[6,8]</sup>

*S. mutans* change the environment of the oral flora, enabling fastidious organisms to colonize and cause the formation of dental plaques.<sup>[9]</sup> It is normally present in low numbers in the plaque of affected individuals. When salivary flow decreases, the pH of the plaque drops, leading to the selection of acid uric (acid tolerant) bacteria such as *S. mutans*.<sup>[10]</sup> This sequence of events indicates that *S. mutans* is involved in the initiation of decay.<sup>[11]</sup>

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Control of plaque can be achieved by mechanical oral hygiene procedures.<sup>[12]</sup> Xylitol can be considered as a safer substance to use than triclosan for the prevention of plaques since triclosan can react with chlorine in tap water and form chloroform and is, hence, considered to be toxic. In addition, xylitol has received an anticariogenic claim approval by the European Food Safety Authority.<sup>[13]</sup>

*P. amboinicus* is also known as Mexican Mint. It is identified as *Coleus amboinicus*, is a semi-succulent perennial plant in the family Lamiaceae with a pungent oregano-like flavor and odor. Its native is Southern and Eastern Africa.<sup>[14]</sup> It acts as the antimicrobial compound.<sup>[15]</sup> Species of *Plectranthus* including *P. amboinicus* has been studied due to its pharmacological properties to validate its popular use.<sup>[15,16]</sup>

The present study is aimed to compare the formation of *P. amboinicus* extract against *S. mutans*.

## MATERIALS AND METHODS

### Test Organisms

The organism *S. mutans* was isolated from saliva sample using special media [Mutans-Sanguis agar] and maintained in Tryptose soya agar at 4°C in the Department of Microbiology, Saveetha Dental College and Hospitals, Chennai, India.

### Methodology

Overnight grown cultures of *S. mutans* from agar plates were inoculated in Tryptose soy broth and incubated at 37°C overnight. Individual wells of sterile polystyrene 96-well flat bottom microtiter plates were filled with 200 µl of culture suspension of the test organism. Uninoculated broth served as negative control. To the wells containing bacterial suspension, different concentrations [10, 20, 40, 80, and 100 µg/ml] of *P. amboinicus* extracts were added and incubated at 37°C for 24 h. After incubation, content in the wells was removed, washed with 0.2 ml phosphate buffer saline to remove free-floating bacteria. The adherence of the bacteria was fixed with sodium acetate (2%) and stained with crystal violet. The crystal violet was removed and 250 µL of acetone was placed in each well to release the crystal violet. Then, finally, the readings were taken. Optical density of stained adherent bacteria was determined with an ELISA reader (Bio-Rad) at wavelength 570 nm. These OD values were taken as index of bacteria adhering the surface and formed biofilm. Experiment was carried out in triplicate and their mean was taken for the analysis.

## RESULTS AND DISCUSSION

In this *in vitro* study, the comparative evaluation of antibiofilm formation activity of *P. amboinicus* extract

against *S. mutans* was done. Figure 1 shows that the picture of *P. amboinicus* leaves and stem of *P. amboinicus* were used for the extraction of essential *P. amboinicus* extract. From Figure 2, we can see that the extract *Plectranthus* is been filled inside the titer plate at different concentrations. Concentrations were 10, 20, 40, 80, and 100. The plate was incubated at 37°C for 24 h. After incubation, content in the wells was removed, washed with 0.2 ml phosphate buffer saline to remove free-floating bacteria. The adherence of the bacteria was fixed with sodium acetate (2%) and stained with crystal violet. The crystal violet was removed and 250 µL of acetone was placed in each well to release the crystal violet.

Figure 3 shows the titer plate with the final result after the above-mentioned staining process. Then, finally, the readings were taken. Table 1 depicts the inhibitory



Figure 1: Visual observation of *Plectranthus amboinicus*

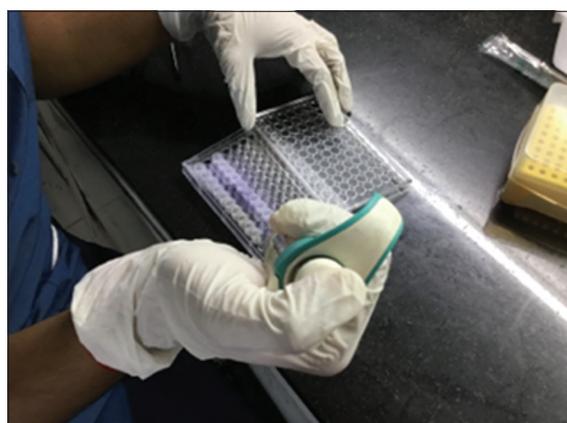


Figure 2: Filling of the polystyrene 96-well titer plate

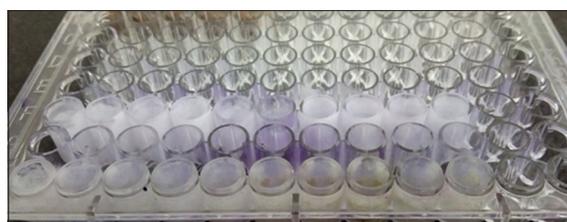


Figure 3: The microtiter plate after staining

**Table 1: Inhibitory concentration**

Concentration of the extract µg/ml	% of inhibition
10	10.27
20	22.45
40	43.75
80	64.25
100	79.43

**Table 2: The OD values taken under ELISA reader**

Mean OD values	Adherence	Biofilm formation
<0.120	Non	Non/weak
0.120–0.240	Moderately	Moderate
>0.240	Strong	High

concentration. We can see that as the concentration of the extract increases with the increase in the percentage of inhibition. It shows that the extract *P. amboinicus* resists the formation of biofilm against *S. mutans*. The Table 1 represents the increase in concentration with the increase in the resistance activity of the extract *P. amboinicus* against *S. mutans*

Table 2 depicts the OD values which were taken as index of bacteria adhering the surface and formed biofilm. It was taken with the help of ELISA reader. It shows that the biofilm formation is moderate under the range of 0.120–0.240. It is clearly seen from Table 1 that the antibiofilm activity of *P. amboinicus* extract against *S. mutans* was found to be more (80%) effective.

Similar studies were carried out on the antibiofilm efficacy of Indian medicinal plant *P. amboinicus* extracts against the biofilm forming *Streptococcus pyogenes* isolated from pharyngitis patients by Manimekalai *et al.*, July 2016.<sup>[17]</sup> Studies were also carried out on efficacy of Thai herbal formula against *S. mutans* by Joycharat N, Limsuwan S, Subhadhirasakul S, Voravuthikunchai SP, Pratumwan S, Madahin I, Nuankaew W, and Promsawat A, in August 2012.<sup>[18]</sup>

## CONCLUSION

Thus, the antibiofilm activity of *P. amboinicus* extract against *S. mutans* was found to be more (80%) effective. Hence, it is evident that it can resist the bacteria in the mouth preventing the formation of dental caries and dental plaque. It acts as the antimicrobial and antibacterial solution. It can be used

as mouthwash in day-to-day life, which is cheap and easily available too.

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