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RESEARCH ARTICLE
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Evaluation of antibacterial potential of Pithecellobium dulce against Streptococcus mutans

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ABSTRACT

Introduction: Pithecellobium dulce is used as traditional medicine. It is being utilized as an antibacterial agent against various oral diseases. Streptococcus Mutans is the major causative bacteria for dental caries. This study aims to evaluate the antibacterial potential of Pithecellobium Dulce on Streptococcus Mutans.

Aim: This study aims to evaluate the antimicrobial activity of Pithecellobium Dulce against Streptococcus Mutans.

Methodology: The minimum inhibitory concentration of Streptococcus mutans was evaluated by the microdilution method utilizing spectrophotometer. The Pithecellobium dulce extract was tested with 25µl, 50 µl, 100 µl concentration, standard and positive control at different time intervals 1hr, 2hr, 3hr, and 4 hr. The minimum bactericidal concentration was evaluated by the agar plate diffusion method at 25µl, 50 µl and 100 µl concentrations and no colonies were counted.

Results: The growth of Streptococcus Mutans bacteria was inhibited and at each concentration (25µl, 50 µl and 100 µl) of the extracts showed significant inhibition in comparison to the positive control. The minimum bactericidal concentration was tested and at 100 µl concentration minimum bacterial count was obtained.

Conclusion: The findings suggest that Pithecellobium dulce extract has potential to be used as an alternative to antimicrobial agent against Streptococcus mutans.

Clinical significance: Antibiotics have been known to cause side effects such as premature death, antibiotic resistance, weakened immune system, and allergy. Hence, there is a need to develop new antibiotics from natural sources

Keywords: *Pithecellobium, antimicrobial, streptococcus mutans, minimum inhibitory concentration, laboratory research*

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INTRODUCTION

Since ancient times, traditional remedies have been used for certain diseases. Nature has an array of inexhaustible compounds with immense potential of healing. In developing countries the use of medicinal plants as traditional medicines is still popular. Pithecellobium dulce belongs to the family of Leguminous plants(1). The plant is generally spread throughout the world but is commonly found in Southeast Asia and India. The leaves are, bipinnate with two sets of leaflets, fruits are generally green when they are unripened and are pink when they ripen and exceptionally sweet in taste, so it is called Jungle jalebi in India and is commonly known as Seema Chinta in southeast Asia(2).

Based on the advanced pharmacological and phytochemical examination and significant results were seen as Antidiabetic, Antifungal, Antitubercular, Antiulcer, anti-hyperlipidemic, anti-inflammatory, and analgesic agent(3). The bark and fruit are used to treat gingival and periodontal diseases, toothache, and bleeding(4).

According to Raghunath et al the micro organisms have developed resistance against many conventional antibiotics by acquisition and expression of resistant genes. Moreover; Antibiotics have been known to cause side effects such as premature death, antibiotic resistance, weakened immune system, and allergy(5).

Hence, there is a need to develop new antibiotics from natural sources. Our team has extensive knowledge and research experience that has translated into high quality publications (6–15). The specific phytochemicals isolated from Pithecellobium Dulce have demonstrated the potential to prevent periodontal diseases by inhibiting bacterial proteolytic enzymes and inflammatory(16)(17) therefore, aim of our study is to validate the antibacterial potential of Pithecellobium Dulce on Streptococcus Mutans which is the major causative organism of Dental caries.

MATERIALS AND METHOD

Sample Preparation

Fresh Pithecellobium dulce fruits were collected from the village Pettaikalipaliyam,Tamil Nadu and were dried under shade for 1 month after segregation of the fruit and seed 50gms of the seed was taken and ground in a coffee grinder to get powder extract.1 gram of powder was taken and added with 100 ml of distilled water and boiled for 15 mins. The extract was further filtered through the sieve of 4.75 µm diameter and the filtrate was further concentrated to 5ml by boiling.

Minimal inhibitory concentration

1. Muller Hinton broth was prepared, sterilized and 6 ml of extract was added to all three test tubes.
2. The bacterial suspension was added to all 5 test tubes in the range of 5×10 5 CFU/ml overnight.
3. The Pithecellobium dulce extract with different concentrations such as (25µl, 50 µl, and 100 µl) was added in three tubes. The fourth tube with sodium fluoride considered as standard and the fifth tube with aerobic suspension which was considered a positive control.(Figure 1)
4. The incubation was done under aerobic conditions at 37°C for varied time intervals 1hr, 2hr, 3hr and 4hr.
5. Then the percentage of dead cells was calculated at a wavelength of 600nm at regular time intervals.

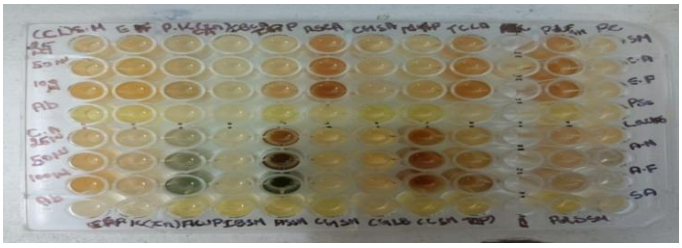

FIGURE 1: The Pithecellobium dulce extract with different concentrations (25µl, 50 µl, and 100 µl) , standard, positive control.

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Minimal bactericidal concentration by Agar dilution method

Muller Hinton Agar was prepared, autoclaved, cooled, and poured onto sterile Petri plates and allowed to solidify. The aerobic suspension with three different concentrations (25µl, 50 µl, and 100 µl) of Pithecellobium dulce extract, standard, and positive control was swabbed on the surface of the aerobic media and was kept in an incubator for 24 hours. After the incubation period, the number of colonies were counted.

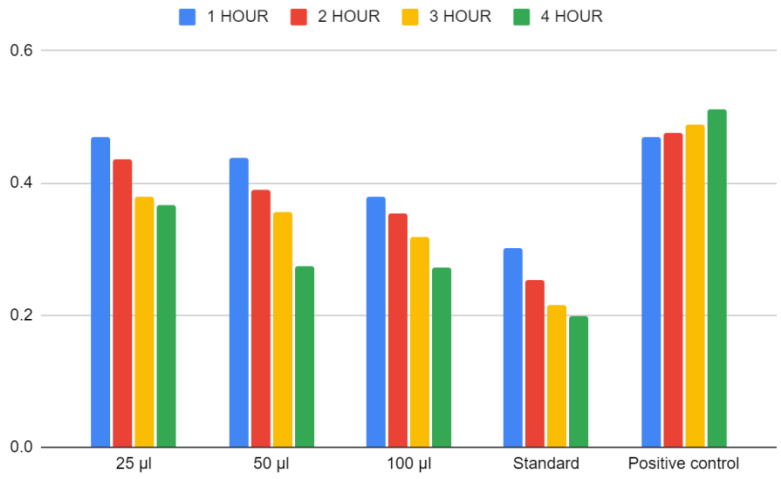
RESULTS

Minimal inhibitory concentration (MIC)

The MIC test showed, the concentration of 25µl, 50 µl, and 100 µl, positive and standard control group, had a significant difference (Table 1). The substantial difference in providing antibacterial potential against Streptococcus mutans bacteria in varied time intervals 1hr, 2hr, 3hr and 4 hr. (Graph 2)

The aqueous crude extract of Pithecellobium Dulce was tested against Streptococcus Mutans. The disc diffusion agar displayed different degrees of growth inhibition. The higher activity was observed against S. Mutans at 100%.

	1 Hour	**2 Hour**	**3 Hour**	**4 Hour**
25 µl	0.470	0.435	0.380	0.366
50 µl	0.438	0.389	0.356	0.275
100 µl	0.379	0.354	0.318	0.272
Standard	0.302	0.254	0.215	0.198
Positive control	0.470	0.475	0.489	0.512



GRAPH 1: Streptococcus mutans activity in varied time intervals 1hr, 2hr, 3hr and 4 hr and at different concentrations (25µl, 50 µl, and 100 µl).

MBC by Agar dilution method

The aqueous crude extract of Pithecellobium Dulce was tested against Streptococcus Mutans on disc diffusion agar and displayed different degrees of growth inhibition. The extract showed higher activity against S. Mutans at 100%.

Concentration	25 µl	50 µl	100 µl
Bacterial colony count	2200	600	120

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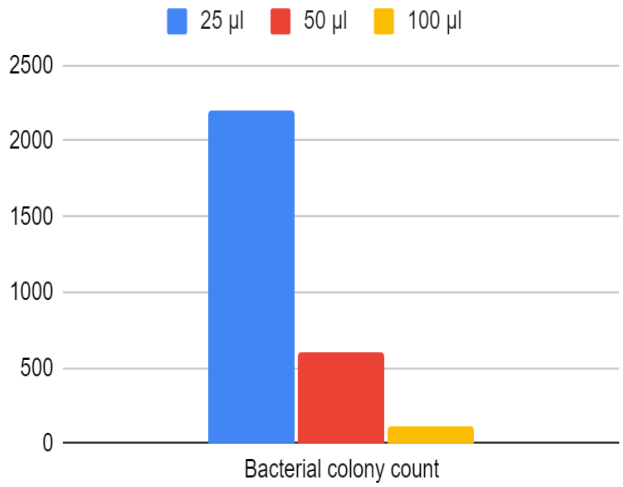

GRAPH 2: The bacteria count at different concentrations (25µl, 50 µl, and 100 µl) of extract

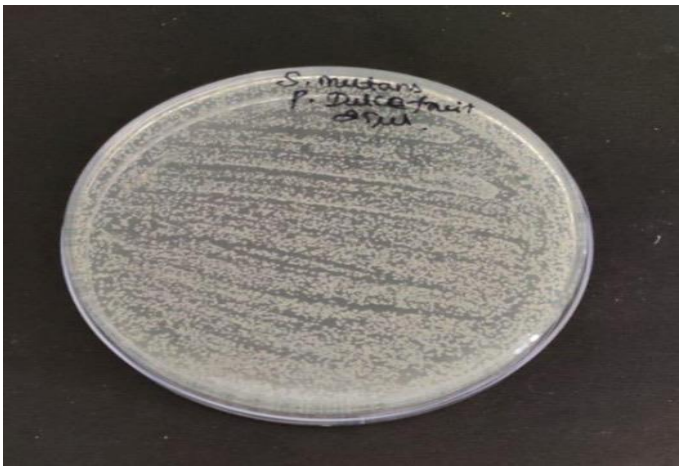

FIGURE 2: Bacterial colony at 25µl concentration

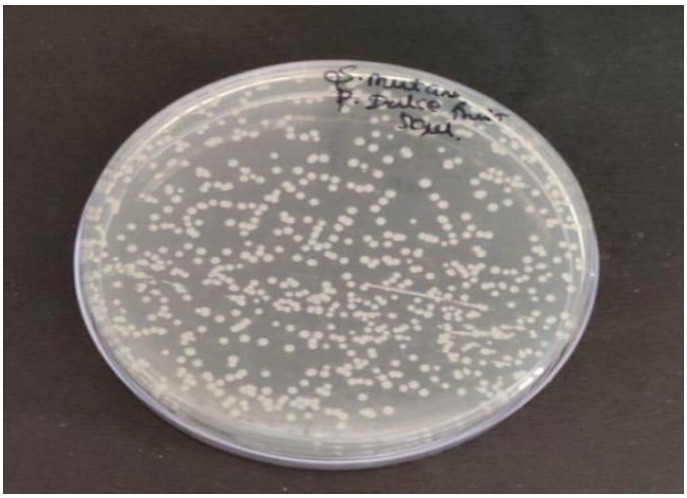

FIGURE 3: Bacterial colony at 50 µl concentration

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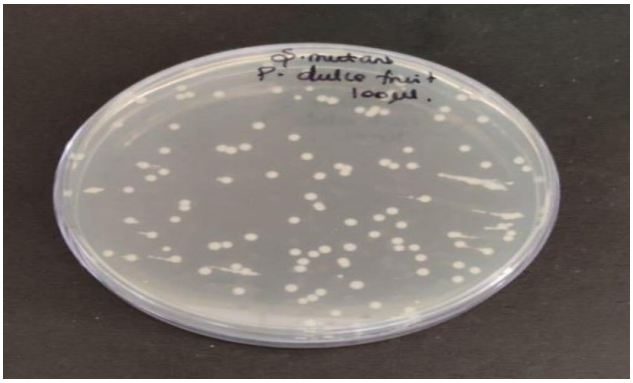

FIGURE 4: Bacterial colony at 100 µl concentration

DISCUSSION

At present the clinicians all over the world are facing a challenge of drug resistance due to evolution of microbes. The use of traditional medicines over conventional medicines has increased by many folds in the past few decades. The predictable tolerance gained by antimicrobial agents increases the desire for the discovery of antimicrobial agents from natural sources(18).

Streptococcus Mutans is the major pathogen responsible for dental caries which has ability to produce biofilms(19).According to our knowledge, currently this is the first study the minimum inhibitory concentration and minimum bacterial concentration of Pithecellobium dulce was evaluated against Streptococcus mutans.

The Minimal Inhibitory concentration (MIC) test of the concentration 100% and 50% with a concentration of 25%, positive and negative controls shows a significant difference. However, the MIC test shows that the concentration of 100% has a significant difference from a concentration of 50%. Therefore it can be concluded that the 100% concentration has a substantial difference in providing antibacterial potential against Streptococcus mutans bacteria.

The minimum bacterial count (MBC) was performed by agar diffusion method. The results of the present work shows the streptococcus colony count was observed to reduce from 2200 to 120 by increasing concentration of crude extract.

Our team has extensive knowledge and research experience that has translate into high quality publications (20–29)

CONCLUSION

Based on the findings and within the scope of the analysis Pithecellobium dulce may be seen as an alternative to antimicrobial agent for clinical use in cariology, but further long-term clinical trials are needed to prove its effectiveness.

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