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Comparing the Antibacterial Efficacy of Morus Alba (Mulberry Leaf) Extract and 0.2% Chlorhexidine: An Invitro Assay

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ABSTRACT

Backgrounds: Morus Alba is being used for the treatment of diabetes to reduce blood sugar level. It has also shown antimicrobial and anti-inflammatory activity against Candida albicans, Saccharomyces cerevisiae, Escherichia coli, Salmonella typhimurium, Staphylococcus epidermis and S. aureus. Extracts of the bark of Morus Alba have also shown bactericidal activity against the oral Streptococci.

Aim: -To compare the antibacterial efficacy of Morus Alba (mulberry leaf) extract with 0.2% chlorhexidine gluconate mouthwash.

Material & methods: The Morus Alba aqueous leaf extraction was carried out by using hot maceration technique with deionised water and the minimal inhibition concentration (MIC) Morus Alba aqueous leaf extract was evaluated against the biofilm forming Streptococcus mutans.

Results: The minimal inhibition concentration (MIC) of hot methanol extract of Morusalba(2.5mg/ 10ml) was used to study its effect on bacterial growth. As the concentration of Morus Alba aqueous leaf extract increasing, the OD values decreases, indicating in reduced bacterial growth. The reduction percentage increases with higher concentration of leaf extract showing a dose-dependent inhibition of streptococcus mutans. The 0.2% CHX shows the highest reduction confirming its effectiveness.

Conclusion: The aqueous extract of Morus Alba mouthwash shows a significant inhibitory effect on streptococcus mutans, with higher concentration leading to greater reduction in bacterial growth.

Keywords: Mulberry leaf Extract, Mouthwash, Aqueous, Antibacterial efficacy

INTRODUCTION:

Dental plaque (biofilm) formation is a naturally occurring process, resulting from bacterial interactions with the acquired salivary pellicle formed over the surface of the tooth shortly after brushing the tooth.[1] Plaque control is utmost essential for the suppression of gingivitis, dental caries, and halitosis-causing microorganisms.[2] Both dental caries and periodontal disease are significantly influenced by the bacteria found in dental plaque or biofilm.[3] Dental caries results from the accumulation of plaque on the surface of the teeth and biochemical activities of complex micro communities. Streptococcus mutans is one of the main opportunistic pathogens of dental caries.[3] Numerous studies have been published that demonstrate the importance of Streptococcus mutans in the development of pit and fissure caries in the primary, mixed, and permanent dentition and the correlation between the presence of S. mutans in saliva

and the number of colonised surfaces. As a result, reducing the amount of S. mutans in the oral cavity would greatly help to reduce the prevalence of dental caries.[4] Dental floss, mechanical or electrical tooth brushing, professional dental scaling, and interdental brushing are the most often utilised instruments in the elimination of supra-gingival plaque.[2] Chemical inhibitors of plaque play an important role in plaque control. A variety of approaches have been considered for chemical plaque control.[5] Vehicles for delivery of chemical agents with anti-plaque/anti-gingivitis action are toothpastes, mouthwashes, spray, irrigators, chewing gum, and varnishes.[5] Mouthwashes are a simple and widely accepted method to deliver the anti-microbial agent (after toothpastes).[5] Although mouth rinses have been used for both medical and cosmetic purposes for centuries, it has only been in recent years that the benefits of using chemical compounds in mouth rinses have been the subject of scientific study and clinical studies.[3] Chlorhexidine gluconate is an antiseptic mouthwash much in demand. Chlorhexidine gluconate mouthwash has served the dental profession over 3 decades. It has remained as a primary agent for chemical plaque control and its clinical efficacy.[5] Hence, there is need of an alternative mouth rinse that could negate all the side effects of chlorhexidine but yet effective equivalent to it.[4] Natural herbs and their varied extracts have been used globally in therapeutic since antiquity.[6] Among many herbal derivatives, Morus alba is one such plant which has garnered great attention because of its anti-oxidative, antidiabetic, antibacterial, antiviral, and anti-inflammatory properties.[7] Morus Alba is being used for the treatment of diabetes to reduce blood sugar level. It has also shown antimicrobial and anti-inflammatory activity against Candida albicans, Saccharomyces cerevisiae, Escherichia coli, Salmonella typhimurium, Staphylococcus epidermis and S. aureus. Extracts of the bark of Morus Alba have also shown bactericidal activity against the oral Strepto-cocci.[8] Leafs of M. alba contain flavonoids such as apigenin (42.7 mg/g) and quercetin (4.0 mg/g). Extracts of the bark of Morus alba have also shown bactericidal activity against the oral Streptococci.[8] The root bark of M. alba exhibits antimicrobial activity against food poisoning.[9] In present study we mainly focus on the inhibitory activity of Morus alba leaf against the biofilm formation by Streptococcus mutans.[8] Hence, the present in vitro-study will be conducted with an aim to compare the antibacterial efficacy of 0.2% chlorhexidine gluconate mouthwash and aquous extract of Morus Alba leaf mouthwash.

MATERIAL & METHODS:

Collection of Morus Alba leaf material:

The medicinal plants used for the evaluation of the antimicrobial study were Mulberry leafs (Morus alba). Fresh leafs of Mulberry Leaf (Morus Alba) were collected from the University of Agricultural and Horticultural Sciences, Bangalore, Karnataka, India, in the month of August 2020. The study was conducted in the central research laboratory of Rajarajeswari Medical College & Hospital.

The leafs of Morus alba shade dried at room temperature and then grounded in an electric grinder. The ground material was passed through a sieve of mesh size 60 to obtain a fine powder, which was used to prepare the extract. The hot extraction procedures were followed to procure crude and partially purified fractions, respectively.

PREPARATION OF AQUEOUS MULBERRY EXTRACTS DRIED POWDER FORM (Hot Maceration)

30 grams (0r 15grams) of mulberry powder were taken in a 250-ml round-bottom flask. After that, 100 mL of deionized water were added to a 250 mL round-bottom flask. Then, the reflux process was performed for 2-3 hours at 60 to 80 degrees C. Later, the reflux apparatus was removed, and the mulberry leaf extract were cooled down to room temperature. The extract was transferred to the 50-ml centrifuge tubes. The extract was centrifuged for 10 minutes at 5000 rpm. After centrifugation, it was transferred to the supernatant in clean, sterile 50-ml centrifuge tubes and stored at 4 °C.

Estimation of the extractive value of the mulberry extract:

Formula:

Extractive value (%) = weight of dried extract/weight of plant material *100

PREPARATION OF AQUEOUS MULBERRY EXTRACTS LIQUID FORM (HOT MACERATION)

15grams of mulberry leaf powder was taken in 150ml of sterile deionized water for extraction

Extractive value calculation:

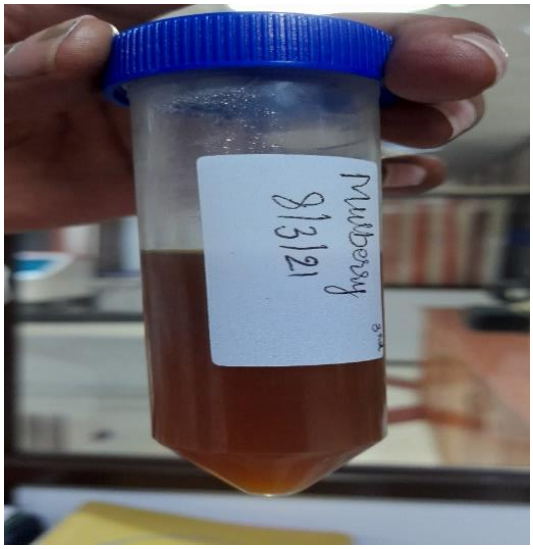
Initial weight of glass petri plate: 41.100g

Average weight of mulberry extract: 0.0684

Extractive value (%) of mulberry aqueous extract is: 0.0684/15gX100

Extractive value (%) = 0.456%

Approximately **0.5%** of mulberry extract in aqueous form obtained.



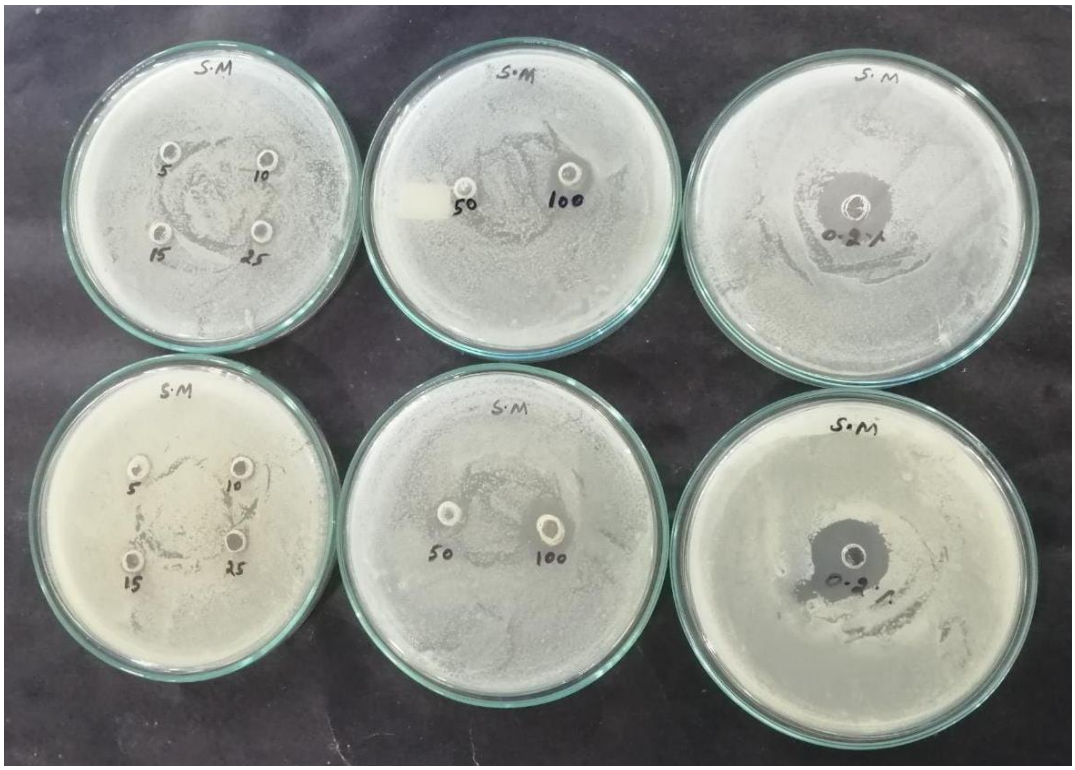
RESULT:

Sl.no	Test Organisms	Strain
1	***Streptococcus mutans***	**MTCC890**
Sl.no	Bacteria	Cfu/ml
1	***Streptococcus mutans***	Mc Farland Standard-0.5

Sl.no	Test organism	Zone of Inhibition (mm)										
		Concentration										
		5	**5**	**10**	**10**	**15**	**15**	**25**	**25**	**50**	**100**	**100**
1	***Streptococcus mutans***	-	-	-	-	-	-	-	-	-	15	13
2	**0.2% chlorhexidine**	20										

TABLE-1:- Shows the inhibition zone for streptococcus mutans using different concentration of Morus alba leaf extract and 0.2% Chlorhexidine

The aqueous extract of Morus Alba shows inhibitory effects against streptococcus mutans at concentrations of 50 and 100 unit, with inhibition zones of 15 and 13 mm respectively. Chlorhexidine used as a control, exhibits a 20 mm inhibition zone. (Table-1)



Antibacterial test against *Streptococcus mutans*

Antibacterial test of liquid sample against *Streptococcus mutans*

The antimicrobial activity of the liquid samples was tested by the agar well-diffusion method. Wells of 6 mm diameter were punched on specific agar media. About 100 µl of pre-cultured test organisms (McFarland Standard-0.5) were spread onto the agar plates. Various concentrations of the liquid sample

were loaded into the wells. **0.2% chlorhexidine** was used *as a positive control for* ***Streptococcus mutans.*** Bacterial plates were incubated at 37 °C for 24 hours, and zones of inhibition were measured and tabulated.

Sl. No	*MIC for Streptococcus mutans*	OD at 600nm			Reduction %
	Concentration 15% [µg/mL]	Trial 1	Trial 2	Avrg.	
	+ve control	0.984	1.002	0.993	
1	10	0.345	0.354	0.349	65.25
2	25	0.213	0.211	0.212	78.65
3	50	0.193	0.193	0.193	80.56
4	100	0.143	0.143	0.143	85.59
5	125	0.139	0.139	0.139	86.00
6	150	0.134	0.136	0.135	86.40
7	175	0.120	0.119	0.119	87.71
8	200	0.111	0.119	0.115	88.41
	0.2% CHX	0.021	0.023	0.022	97.78

MIC (Minimum inhibitory concentration)

The minimum inhibitory concentration of the sample was determined by using Streptococcus ***mutans***. The table shows the minimum inhibitory concentration for streptococcus mutans indicating the effectiveness at various concentration of aqueous extract of Morus Alba mouthwash agent in inhibiting the growth of the bacteria. The optimal density (OD) at 600nm is used as a measure of bacterial growth with lowest OD values indicating less growth.

A. Effect of aqueous extract of Morus Alba mouthwash at different concentrations:

- 10µg/ml: The OD decrease to an average of 0.349, indicating a reduction in bacterial growth by 65.25%.
- 25µg/ml: The OD decrease to an average of 0.212, indicating a reduction in bacterial growth by 78.65%.
- 50µg/ml: The OD remains at0.193, showing an 80.56% reduction.
- 100µg/ml: The OD drops at 0.143, showing an 85.59% reduction.
- 125µg/ml: The OD is 0.139, showing an 86.00% reduction.
- 150µg/ml: The OD is at 0.135, showing an 86.40% reduction.
- 175µg/ml: The OD is at 0.119, showing an 87.71% reduction.
- 200µg/ml: The OD is 0.115, showing an 88.41% reduction

B. 0.2% CHX (Chlorhexidine): the OD for 0.2% CHX is very low at 0.022, indicating a 97.78% reduction in the bacterial growth. This shows CHX is highly effective in inhibiting streptococcus mutans.

DISCUSSION:

India is a land filled with nature's medicinal plants. Herbal extracts are known to possess antimicrobial compounds, especially against bacterial pathogens. Extracts of Morus Alba leafs provides antimicrobial potential against harmful microorganisms. The strategies for effective treatment against the dental caries

includes the elimination of mutans streptococci, inhibition of the colonization, reduction of the glucosyltransferase activity, inhibition of glucan production. Many studies shown that 0.2% chlorhexidine containing mouthrinse (Corsodyl) had maximum antimicrobial activity.[10] On the other hand, few studies have also shown that 0.20% chlorhexidine is better than 0.12% of chlorhexidine mouthrinse.[11] In present study, we tested the aqueous extract of Morus Alba leaf against such biofilm and compared with 0.20% chlorhexidine. The minimal inhibition concentration (MIC) of hot methanol extract of Morus Alba (200ug/ ml) was shown in complete inhibition glucosyltransferase production, reduction of the glucosyltransferase activity in terms of glucan production, reduction in the bacterial density of the biofilm and the decrease in thickness of the preformed biofilm of Streptococcus mutans.[1

The study done by Rathore et al shown that Neem bark was found to possess more significant antibacterial activity than Neem leafs and Tulsi leafs.[6] In study done by Lokegaonkar S. P et al, the minimum biofilm inhibitory concentration of hot ethanolic extract of Morus alba for S.mutans and S.sanguinis was found to be 15mg/ml.[8] Pawithra P et al. conducted a research in which they found that the ethanolic extract of M. alba leafs, with MICs of 0.14 and 5.01 mg /ml, respectively, had a more powerful antibacterial activity against S. mutans.[12]

In Islam Barira et al study the purified compound of M. alba showed an 8-fold greater reduction of MIC against S. mutans than the crude extract (MICs, 15.6 and 125 mg/L, respectively) and the extract strongly inhibited biofilm formation of S. mutans at its active accumulation and plateau phases.[13 Various studies have been conducted where herbal extracts were tested for their antibacterial potential against the dental plaque forming microorganisms. Psidium cattleianum (Brighenti et al 2008), Eucalyptus globules (Osawa et al 1995), Rhizoma rhei, Semen areca, Rhizoma ligustici chuanxiong and Catechu (Xiao et al 2004), P. corylifolia (Kastura et al 2001) were studied for their antibacterial activity against the S.mutans, while very limited data was available about their effects against the biofilm.[14,15,16,17]

CONCLUSION:

The aqueous extract of Morus Alba mouthwash shows a significant inhibitory effect on streptococcus mutans, with higher concentration leading to greater reduction in bacterial growth. The agent's effectiveness plateaus at around 100µg/ml, beyond which further increases in concentration providing diminishing result. The comparison with CHX demonstrates that while the aqueous extract of Morus Alba mouthwash is effective, CHX is better in terms of bacterial inhibition.

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