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Potential of Mangifera Indica as an Anti-Biofilm Agent In Preventing Dental Caries

Dr. Shilpa B Mendon Ph.D
(Clinical Annotation Specialist, WebMD, India)

Abstract: The aqueous and alcoholic extract of Mangifera indica was tested for its ability to inhibit the growth and some physiological functions of Streptococcus mutans. The extract failed to exhibit any antibacterial activity in vitro even when tested at concentrations as high as 300 mg/ml. The extract strongly inhibited sucrose induced adherence and glycolysis of salivary bacteria. The aqueous and alcoholic extracts significantly decreased adherence to glass by 88% and 84%, respectively, at a concentration of 20 mg/ml ($p < 0.05$). Only the alcoholic extract inhibited sucrose-induced adhesion to the tooth surface at all tested doses. In vitro salivary glycolytic process was significantly inhibited by both the alcoholic and aqueous extracts at doses of 40, 80, and 100 mg/ml. As M. indica can inhibit the metabolism and adherence of S. mutans without unbalancing the oral flora, it is an effective anti-biofilm agent that can prevent dental caries with care.

Keywords– Mangifera indica, Streptococcus mutans, Dental caries,

I. INTRODUCTION

Despite being a preventable condition, dental caries affects between 60 and 90 percent of people worldwide [1]. Research by the European Federation of Periodontology (EFP) estimates that dental caries treatment costs US$357 billion (€331 billion) annually for individuals aged 12 to 65 worldwide. According to the study, tooth loss, severe periodontitis and dental cavities cost $188 billion in lost productivity each year [2].

Preventing dental caries is essential to managing public health. Steps proposed to prevent dental caries include balancing the levels of oral bacteria, controlling the consumption of sugary and starchy foods, strengthening the demineralized enamel though fluoridated products Among these strategies, improvement of oral flora is the most effective in preventing dental cavities [3]. The accumulation of pathogenic biofilm is thought to be the main cause of dental caries because the disease process starts in the biofilm that covers the tooth surface [3-5]. Dental caries is essentially a biofilm-induced disease, rather than an infectious disease [6]. Streptococcus mutans plays a key role in transitioning the biofilm from being non-pathogenic to cariogenic. S. mutans converts dietary sucrose to extracellular polysaccharides (EPS), glucan, by using glucosyltransferases adsorbed to saliva-coated tooth enamel surfaces. The insoluble glucans supply a matrix that holds the microbial cells together to form structurally cohesive cell clusters known as microcolonies. Elevated amounts of insoluble EPS are associated with higher bacterial adhesion and biofilm cohesiveness. The fructose moiety from sucrose gets metabolized to acid, which lowers the pH at the tooth-biofilm interface causing the enamel to demineralize [3,7-8]. Chemical agents with anti-biofilm properties viz. chlorhexidine, delmopinol and triclosan are effective in preventing dental caries as they inhibit the metabolism and adherence of bacterial cells [9-11]. Limitation with chemical agents include, unbalancing oral flora, limited penetration and side effects such as tooth staining, mucosal desquamation [12]. There is a need to explore natural agents as preventive measures for dental caries [3]. In southern India, the leaves of the Mangifera indica plant are used to treat gum disease and tooth decay. When using mango leaf to clean dental enamel, it is advised to roll the leaf into a cylinder with the glossy side inside, bite off one end, and brush with that end [13-15]. It was therefore of interest to provide new insights into the mode of action of M. indica in the oral cavity.

II. MATERIAL AND METHODS

2.1 Culture of the micro-organism and preparation of the cell suspension

The microbe used in this investigation, S. mutans were isolated from a healthy human volunteer's tooth plaque. After being isolated onto plates of Mitis salivarius (MS) agar, the strain from the human dental plaque was cultured anaerobically for 24 hours at 37oC. Colonies of S. mutans were removed into fluid thioglycolate media and incubated for 18 hours at 37oC to create a cell suspension.

2.1 Source of the plant material and preparation of the plant extract

The fresh leaves of M. indica was obtained from the local market. After air drying, a determined amount was suspended in ten times its volume of sterile distilled water (or, in the case of an alcoholic extract, 50% v/v methanol) and allowed to sit at 4oC for 72 hours. The aqueous and alcoholic extract was decanted, filtered through a muslin cloth to clarify it, and then evaporated in a porcelain dish with a flat bottom at 4oC. To achieve a final concentration of 30% w/v, the extract was suspended in 400 ml of polyethylene glycol (PEG) (20% v/v) and sterile distilled water. Concentrations of 2, 4, 8, 10, 15, 20, 25 and 30% w/v were obtained by diluting the concentrated extract with sterile diluted water.

2.3. Antimicrobial activity of the extract

The 18-hour-old culture of S. mutans was mixed with brain heart infusion (BHI) agar and set in petriplates (0.1 ml of the culture fluid for every 10 ml of agar). Disks of sterile filter paper, each 6 mm in diameter, were put on the agar after being impregnated with known extract dilutions. Zone of inhibition produced with different extract concentrations were measures after 24 hours of incubation at 37oC.

2.4. Adherence inhibition

Overnight culture of S. mutans was inoculated with BHI broth containing 2% sucrose and 0.1 ml of different concentrations of the extract (ranging from 2mg/ml to 10mg/ml). Cells cultured in BHI broth with sucrose and 0.1 ml of 20% v/v PEG 400 made up the control. After being angled at 30°, each tube was incubated for 24 hours at 37°C. The adherent and nonadherent bacteria were quantitated spectrophotometrically at 540 nm [16].

For examining the impact of the extract's different concentrations on adhesion of S. mutans to the tooth's surface, saliva-coated sterile human teeth were exposed to 1 ml of the different extract concentrations (ranging from 2 to 10%) for one minute. Teeth in contact with 1 ml of PEG 400 (20% v/v) served as the control. After that, the teeth were placed in 5 ml of BHI broth with 2% sucrose, inoculated with S. mutans overnight cultures, and incubated for 24 hours at 37oC. Using spectrophotometry, the adherent bacteria (cells that stuck to the tooth and glass surface) and nonadherent bacteria were measured at 540 nm [17].

2.5. Inhibition of glycolysis

The effect of the extracts on acid production was studied by two in vitro methods:

To 2 ml of fresh clarified human saliva was added 0.1 ml 5% glucose and 0.1 ml of the various concentrations of the extracts (from 2 to 10%). Control consisted of the saliva glucose mixes containing 0.1 ml PEG 400 (20% v/v). The pH of the saliva glucose mixes was recorded immediately and at 60 min intervals for the next 5 h [18].

5 ml BHI broth containing 2% sucrose and 0.1 ml of various concentration of the extracts (from 2 to 10%) were inoculated with the overnight cultures of S. mutans and incubated at 37oC for 24 h. The pH of the bacterial broth was recorded at the onset and after 24 h incubation [19]

III. STATISTICAL ANALYSIS

Data were analyzed for inhibition of adherence and acid production. For in vitro adherence, data from the test and the control groups were compared using Student's t-test. For in vitro glycolysis, data from the test at various time intervals were compared with the data from the control at the same time interval using Student's t-test. All values were considered significant when $P<0.05$.

IV. RESULTS

4.1. Antimicrobial activity against S. mutans

The aqueous and alcoholic extract of M. indica showed no zone of inhibition for any of the concentrations tested (Table1).

4.2. Adherence Inhibition

In vitro adhesion of S. mutans to glass was inhibited when cells of S. mutans were cultured in BHI broth with sucrose and different quantities of the aqueous and alcoholic extract of M. indica, (Table 2). At concentration of 20 mg/ml, the aqueous extract caused 88% inhibition and the alcoholic extract caused 84% inhibition to glass surface. The alcoholic extract was also effective in inhibiting adherence to tooth at concentration of 20 mg/ml and higher. The aqueous extract did not produce comparable outcomes at any of the tested concentrations.

4.3. Glycolysis Inhibition

The in vitro salivary glycolytic assay results showed that at concentrations of 40 mg/ml, 80 mg/ml and 100 mg/ml, of both the aqueous and alcoholic extract, the pH values of saliva glucose mixes at the fifth hour into the glycolytic reaction were considerably higher than control samples (Table 3 and4). But there was no evidence of a long-lasting effect. The pH after 24 hours was significantly lower than the control for all concentrations tested with both aqueous and alcoholic extract (Table 5).

V. DISCUSSION

Dental caries forms only when bacteria in the plaque on tooth surfaces produce sticky, insoluble glucan from sucrose, which strengthens their adherence to the tooth surface, and when sucrose ferments to lactic acid, which builds up and causes localized decalcification of tooth enamel. Since dental biofilm plays a significant role in the ethology of dental caries, preventative efforts should focus on managing it [20]. The current study shown that both alcoholic and aqueous extracts of M. indica can prevent dental caries from forming because they significantly reduced the adhesion caused by sucrose and, as a result, biofilm cohesiveness. The study's findings also indicate that M. indica extract dramatically decreased salivary bacterial glycolysis, which could stop dental enamel from decalcifying.

Clinical investigations using mouthwash containing M. indica leaves showed similar outcomes. In a clinical study with 20 children aged 8 to 14, the effects of mouthwash containing extract of M. indica leaves and chlorhexidine on salivary S. mutans count and plaque status were compared. It was discovered that both mouthwashes promoted healthier gingival tissue, improved plaque control, and dramatically reduced bacteria counts [21]. After washing with M. indica extract, 65% of participants in another randomized crossover trial with 20 students ages 20 to 25 reported a decrease in microorganisms and a significant increase in salivary pH ($p < 0.000$) compared to the baseline value [22].

Thus, it can be postulated that the physiological mechanisms in S. mutans that cause localized tooth surface degradation are inhibited by M. indica extract. These observations are made in both in vitro and in vivo studies.

Most importantly, the results of the study lend credence to the conventional method of utilizing mango leaves to clean dental enamel. Throughout the many years that this method has been used, no adverse side effects have ever been reported.

. VI. CONCLUSION

Caries prevention should concentrate on the effective removal of plaque from the tooth surfaces during daily oral care since dental caries is the result of localized tooth surface degradation caused by metabolic processes occurring in the biofilm (dental plaque) covering the afflicted area. Plaque clearing in the caries process has received less attention in preventive measures because all caries prevention treatments concentrate only on the remineralization of the tooth surface. Natural products, such as M. indica, may be recommended for preventing dental caries because they are effective anti-biofilm agents and have less side effects than synthetic antimicrobials.

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TABLE 1: Effect of Aqueous and Alcoholic Extract of *M. indica* on Growth of *S.mutans*

Extract	Diameter of Zone of Inhibition in mm for various concentration								
	20 mg/ml	40 mg/ml	60 mg/ml	80 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	250 mg/ml	300 mg/ml
Aqueous *M.indica*	0	0	0	0	0	0	0	0	0
Alcoholic *M.indica*	0	0	0	0	0	0	0	0	0
Values are expressed as the Mean ± S.E.M n=10									

TABLE 2: Effect of Aqueous and Alcoholic Extract of *M. indica* on Adherence of *S. mutans*

Extract	**Concentration**	**% inhibition of adherence to glass (n= 10)**	**% inhibition of adherence to teeth & glass (n=5)**
Aqueous *M.indica*	Control		
	20 mg/ml	87.78 ±1.56*	16.48 ± 5.23[a]
	40 mg/ml	94.8 ± 1.75*	17.23 ± 5.72 [a]
	80 mg/ml	97.6 ± 0.39*	12.2 ± 5.28[a]
	100 mg/ml	98.01 ± 0.41*	17.06 ± 5.86 [a]
Alcoholic *M.indica*	Control		
	20 mg/ml	84.15 ± 4. 01*	56.64 ± 9.51*
	40 mg/ml	97.42 ± 0.32*	48.94 ± 8.89*
	80 mg/ml	97.4 ± 0.58*	56.24 ± 6.03*
	100 mg/ml	95.1 ± 2.66 *	46.18 ± 8.59*
Values are expressed as the Mean ± S.E.M **significant difference at $p < 0.05$* [a] *non-significant difference between the control and treated group*			

TABLE 3: Temporal Effects of Aqueous Extract of *M. indica* on *In vitro* Salivary Glycolysis of *S. mutans*

Incubation Time in Hours	**pH values of the saliva glucose mixes of control**	**pH values of the saliva glucose mixes containing various concentration of extract**			
		20 mg/ml	**40 mg/ml**	**80 mg/ml**	**100 mg/ml**

0	6.64 ± 0.02	6.22 ± 0.01*	5.98 ± 0.01*	5.69 ± 0.05*	5.40 ± 0.05*
1	6.07 ±0.04	5.61 ± 0.05*	5.51 ± 0.09*	5.41 ± 0.05*	5.31 ± 0.05*
2	5.44 ±0.05	5.23 ± 0.04*	5.22 ± 0.04*	5.2 ± 0.02*	5.13 ± 0.01*
3	5.04 ±0.03	4.72 ± 0.03*	4.71±0.03*	4.91 ± 0.04[a]	5.06 ± 0.09 [a]
4	4.73 ±0.05	4.47 ± 0.02*	4.52 ±0.06*	4.73 ± 0.01 [a]	4.94 ± 0.02*
5	4.32 ±0.01	4.28 ± 0.02 [a]	4.68 ±0.03*	4.68 ± 0.03*	4.85 ± 0.07*

Values are expressed as the Mean ± S.E.M
n= 3
**significant difference at $p < 0.05$*
[a] *non-significant difference between the control and treated group*

TABLE 4: Temporal Effects of Alcoholic Extract of *M. indica* on *In vitro* Salivary Glycolysis of *S. mutans*

Incubation Time in Hours	pH values of the saliva glucose mixes of control	pH values of the saliva glucose mixes containing various concentration of extract			
		20 mg/ml	40 mg/ml	80 mg/ml	100 mg/ml
0	6.87 ± 0.04	6.7 ± 0.01*	6.27 ± 0.01*	6.3 ± 0.02*	6.25 ± 0.05*
1	6.31 ± 0.04	6.27 ± 0.01 [a]	6.11 ± 0.01*	6.11 ± 0.005*	6.13 ± 0.02*
2	5.55 ± 0.04	5.67 ±0.03 [a]	5.67 ±0.04 [a]	5.74 ± 0.06 [a]	5.83 ± 0.05*
3	5.04 ± 0.17	4.82 ± 0.05 [a]	5.45 ± 0.1 [a]	5.33 ± 0.06 [a]	5.68 ± 0.07*
4	4.51 ± 0.08	4.44 ± 0.05 [a]	5.05 ±0.07*	4.86 ± 0.06*	5.13 ± 0.11*
5	4.23 ± 0.04	4.27 ± 0.05 [a]	4.86 ± 0.06*	4.51 ± 0.12 [a]	4.86 ± 0.04*

Values are expressed as the Mean ± S.E.M
n= 3
**significant difference at $p < 0.05$*
[a] *non-significant difference between the control and treated group*

TABLE 5: Effect Of Aqueous and Alcoholic Extract of *M. indica* on Acid Production by *S. mutans* after 24 Hours Incubation

Extract	Concentration	pH at onset	pH after 24 hours incubation
Aqueous *M.indica*	Control	7.15 ± 0.006	4.315 ± 0.0042
	20 mg/ml	7.105 ± 0.002	4.178 ± 0.012*
	40 mg/ml	7.106 ± 0.008	4.198 ± 0.008*
	80 mg/ml	7.08 ± 0.002	4.258 ± 0.014*
	100 mg/ml	7.07 ± 0.002	4.29 ± 0.0136 [a]
Alcoholic *M.indica*	Control	7.16 ± 0.004	4.38 ± 0.006
	20 mg/ml	7.17 ± 0.01	4.23 ± 0.015 [a]
	40 mg/ml	7.15 ± 0.007	4.24 ± 0.012 [a]
	80 mg/ml	7.11± 0.006	4.27 ± 0.01 [a]
	100 mg/ml	7.09 ± 0.011	4.33 ±0.016*

Values are expressed as the Mean ± S.E.M
n= 6
**significant difference at $p < 0.05$*
[a] *non-significant difference between the control and treated group*

REFERENCES

[1] F. Veneri, S.R. Vinceti, T. Filippini, Fluoride and caries prevention: a scoping review of public health policies, Ann Ig, 36 (3), 2024, 2024 270-280.
[2] J. Booth, EFP reveals stunning global cost of gingivitis, caries, tooth loss, Dental Tribune, 2024. Available at: https://www.dental-tribune.com/news/efp-reveals-stunning-global-cost-of-gingivitis-caries-tooth-loss/, Accessed April 17, 2025

[3] X. Chen, E.B. Daliri, N. Kim, J.R. Kim, D. Yoo, D.H. Oh, Microbial Etiology and Prevention of Dental Caries: Exploiting Natural Products to Inhibit Cariogenic Biofilms, Pathogens, 9(7), 2020, 569.

[4] S. Takenaka, T. Ohsumi, Y. Noiri, Evidence-based strategy for dental biofilms: Current evidence of mouthwashes on dental biofilm and gingivitis, Jpn. Dent. Sci. Rev, 55, 2019, 33–40.

[5] K. Yadav, S. Prakash, Dental Caries: A microbiological approach, J. Clin. Infect. Dis. Pract, 2, 2017, 1–5.

[6] C.P.C. Sim, S.G. Dashper, E.C. Reynolds, Oral microbial biofilm models and their application to the testing of anticariogenic agents, J. Dent, 50, 2016, 1–11.

[7] W.H. Bowen, H. Koo, Biology of Streptococcus mu tans -derived glucosyltransferases: role in ex tracellular matrix formation of cariogenic biofilms, Caries Res, 45, 2011, 69–86.

[8] R.G. Quivey, W.L. Kuhnert, K. Hahn, Adaptation of oral streptococci to low pH, Adv Microb Physiol, 42, 2000, 239–274.

[9] S. Takenaka, T. Ohsumi, Y. Noiri, Evidence-based strategy for dental biofilms: Current evidence of mouthwashes on dental biofilm and gingivitis, Jpn. Dent. Sci. Rev, 55, 2019, 33–40.

[10] J. Neilands, U. Troedsson, T. Sjodin, J.R. Davies, The effect of delmopinol and fluoride on acid adaptation and acid production in dental plaque biofilms, Arch. Oral Biol, 59, 2014, 318–323.

[11] B. Stewart, T.A. Shibli, M. Araujo, L.C. Figuciredo, F. Panagakos, et al, Effects of a toothpaste containing 0.3% triclosan on periodontal parameters of subjects enrolled in a regular maintenance program: A secondary analysis of a 2-year randomized clinical trial, J. Periodont, 91, 2020, 596–605.

[12] Y. Sakaue, S. Takenaka, T. Ohsumi, H. Domon, Y. Terao, Y. Noiri, The effect of chlorhexidine on dental calculus formation: An in vitro study, BMC Oral Health, 18 (1), 2018, 52.

[13] Trinity Phix, Toothbrush Alternatives: More Ways to Clean Your Teeth Naturally, Available at: https://www.trinityphix.com/toothbrush-alternatives-natural-toothbrush, Accessed April 17, 2025

[14] 5 Effective Ayurvedic tips that can keep the dentist away. Available at: https://www.artofliving.org/in-en/ayurveda/tips/undying-smile-ayurvedic-way, Accessed April 17, 2025

[15] Y. Salimi, N. Tavahodi, H. Taheri, M. Masoudi, M.S. Modaber, N. et al, Effect of Mangifera Indica (Mango) on Dental Caries: A Systematic Review, Nutr Metab Insights, 16, 2023, doi: 10.1177/11786388231204200.

[16] Segal R, S. Pisanty, R. Wormser, E. Azaz, M.N. Sela, Anticariogenic activity of licorice and glycyrrhizine I: Inhibition of in vitro plaque formation by Streptococcus mutans, J Pharm Sci, 74(1), 1985, 79-8,

[17] R.T. Evans, P.J. Baker, R.A. Coburn, R.J. Genco, Comparison of antiplaque agents using an in vitro assay reflecting oral conditions, J Dent Res, 56(6), 1977 Jun;56(6):559-67.

[18] A.M. Corner, V.J. Brightman, S. Cooper, S.L. Yankell, D. Malamud, Clinical study of a C31G containing mouthrinse: effect on salivary microorganisms, J Clin Dent, 2(2), 1990, 34-38.

[19] J.E. Ciardi, A.B. Rosenthal, W.H. Bowen, Rapid Quantitative Determination of the Effect of Antiplaque Agents and Antisera on the Growth, Acid Production, and Adherence of Streptococcus mutans, Journal of Dental Research, 60(3), 1981, 756-762.

[20] F. Meyer, E. Schulze Zur Wiesche, B.T. Amaechi, H. Limeback, J. Enax, Caries Etiology and Preventive Measures, Eur J Dent, 18(3), 2024, 766-776.

[21] S.S. Bhat, K.S. Hegde, C. Mathew, S.V. Bhat, M. Shyamjith, Comparative evaluation of Mangifera indica leaf mouthwash with chlorhexidine on plaque accumulation, gingival inflammation, and salivary streptococcal growth, Indian J Dent Res, 28(2), 2017, 151-155.

[22] G. Sharma, S. Sunitha, C. Reddy, Influence of Salvadora persica (Miswak) and Mangifera indica (Mango) extracts on Streptococcus mutans and salivary pH - A comparative study, J Indian Assoc Public Health Dent, 8, 2010, 116-120.

[23] S.G. Karkera, Screening and Evaluation of Phytochemicals for Dental Use, M. Pharm diss., Bombay College of Pharmacy, Mumbai University, India, 1998