ORIGINAL RESEARCH

Antibiofilm Efficacy of *Mimosa pudica* against Clinical Isolates of *Streptococcus mutans* as a Mouthrinse

Baskar Kavya[1], Nagaraj NJ[2], Manavalan Madhana Madhubala[3], Sekar Mahalaxmi[4]

Received on: 29 April 2022; *Accepted on:* 01 June 2022; *Published on:* 26 August 2022

Abstract

Aim: The aim of this study was to comparatively evaluate the antibiofilm efficacy of *Mimosa pudica* L. and chlorhexidine (CHX) against *Streptococcus mutans* (*S. mutans*) as a mouthrinse.

Methods: *S. mutans* were cultured from saliva collected from high caries risk patients and inoculated in Mueller–Hinton agar plates. The mouthrinses were grouped as follows: group I—10% aqueous extract of *M. pudica* L. mouthrinse, group II—0.2% CHX mouthrinse, and group III—saline. The resazurin-based dye microbroth assay was used to check the effect of test rinses on the redox activity of salivary bacteria and optical density (OD) values were recorded. Biofilm was grown on the surface of enamel slabs for 7 days in an anaerobic work station using *S. mutans* and treated with respective mouthrinses for 2 minutes at room temperature. The slabs were stained using fluorescent dye propidium iodide and SYTO9 and visualized under confocal scanning laser microscopy (CLSM) and qualitatively analyzed for live/dead bacteria. The percentage of viability was determined and statistically analyzed using the one-way analysis of variance (ANOVA) test at the *p*-value of <0.05.

Results: Based on OD values of resazurin-based dye assay and CLSM analysis, group II showed higher antibacterial efficacy with significant differences than groups I and III.

Conclusion: The percentage of biofilm inhibition was found to be less for aqueous extract of *M. pudica* L. than CHX. However, it is evident that *M. pudica* L. can resist the oral cariogenic bacteria, thereby preventing the formation of dental caries.

Keywords: Biofilm, Chlorhexidine, *Mimosa pudica* L., Mouthrinse.

World Journal of Dentistry (2022): 10.5005/jp-journals-10015-2097

Introduction

Dental plaque is a biofilm that covers most of the oral cavity surface. The succession of dental plaque development can lead to dental caries, gingivitis, and periodontitis. Dental caries is the most multifactorial oral disease among mankind. It occurs mainly due to the microbiological shifts within the complex biofilm present on the tooth surface.[1] Even though the etiology remains highly complex with salivary factors and dietary sugars, microorganisms and their preventive therapy, play a major role in the progress of dental caries.[2]

S. mutans are considered as the most cariogenic microorganisms in dental biofilm due to their capacity to synthesize extracellular polysaccharides (EPS) in addition to its acid-tolerant and acidogenic characteristics. They initiate plaque formation by synthesis of water-insoluble glucan from sucrose by action of glucosyltransferase.[3] EPS promote bacterial adherence to the tooth surface and contribute to the structural integrity of dental biofilms leading to enamel demineralization.

Effective prevention of dental caries can be achieved by mechanical removal of dental plaque through proper brushing and flossing. However, the majority of the population may not perform mechanical plaque removal efficiently; the use of antibacterial mouthrinses may help to maintain oral hygiene by inhibiting the regrowth and reattachment of bacteria onto the enamel.[4]

Chlorhexidine, 1,1′-hexamethylene-bis-[5-(4-chlorophenyl)-biguanide], (CHX) is a cationic antiseptic used in different medical fields due to its broad spectrum of antibacterial action. The compound is a strong base and is available in the form of digluconate. CHX has also been the effective mouthwash of choice owing to its dramatic therapeutic effect on oral biofilms and oral substantivity. However, owing to its undesirable side effects like staining of teeth, altered taste sensation, toxic effects on connective tissues, dryness and soreness of oral cavity, there is always a quest for nontoxic herbal resources. Moreover, the antibiotic resistance which is emerging during the last decade paves a way with alternative antimicrobial therapies. Especially, bacterial resistances toward triclosan or quaternary ammonium compounds and CHX have to be focused with great attention on oral health care. The mechanisms conferring resistance toward CHX include multidrug efflux pumps and cell membrane changes.[5]

With a growing tendency to "go natural," various herbal extracts are being widely explored as an alternative to synthetic mouthwashes. There are abundant evidences available on the use

[1]Department of Conservative Dentistry and Endodontics, Indira Gandhi Institute of Dental sciences, Sri Balaji Vidyapeeth, Puducherry, India

[2]Department of Conservative Dentistry and Endodontics, Rajas Dental College, Chennai, Tamil Nadu, India

[3,4]Department of Conservative Dentistry and Endodontics, SRM Dental College, SRM Institute of Science and Technology, Chennai, Tamil Nadu, India

Corresponding Author: Manavalan Madhana Madhubala, Department of Conservative Dentistry and Endodontics, SRM Dental College, SRM Institute of Science and Technology, Chennai, Tamil Nadu, India, Phone: +91 9994482281, e-mail: madhanam@srmist.edu.in

How to cite this article: Kavya B, NJ N, Madhubala MM, *et al.* Antibiofilm Efficacy of *Mimosa pudica* against Clinical Isolates of *Streptococcus mutans* as a Mouthrinse. World J Dent 2022;13(6):582–586.

Source of support: Nil

Conflict of interest: None

of plants and plant products in preventing caries.[6] *M. pudica* L. is a creeping perennial flowering also called as "touch me not or sensitive plant, sleepy plant." It belongs to pea/legume family, Fabaceae and Magnoliopsida taxon which has recently received attention of researchers worldwide for its pharmacological activities. It has antimicrobial, antidiabetic, anti-inflammatory, antioxidant, and wound healing activities.[7] It has been reported to contain mimosine (an alkaloid), free amino acids, sitosterol, linoleic acid, and oleic acid and is also proved to be active against oral pathogens.[8] Joshi and Joshi, in an earlier study showed that the leaf powder of *M. pudica* L. when mixed with water and orally rinsed relieved pain in tooth and gum.[9]

As there are no literature reports on the antibacterial efficacy of *M. pudica* L. against *S. mutans* biofilms, the aim of this study was to comparatively evaluate the antibacterial and antibiofilm efficacy of *M. pudica* L. and CHX as mouthrinses against *S. mutans*.

Methods

Preparation of the Aqueous Extract of *M. pudica* L.

Leaves of *M. pudica* L. were obtained from a local plant nursery in Chennai, India. It was sundried in shade and then powdered with the help of mortar and pestle. The powder was suspended in sterile distilled water 10 times of its quantity in a round-bottomed flask. It was kept at 4°C for 72 hours. Then, the aqueous extract was decanted, clarified by filtration through a muslin cloth, and evaporated in a flat-bottomed porcelain dish at 40°C. The 100 gm of dried extract was again suspended in polyethylene glycol and 200 mL of distilled water which was evaporated to get the final concentrate. It was then diluted with sterile distilled water to get a mouthrinse of 10% (w/v) concentration.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the crude leaf extract against *S. mutans* was determined by the broth tube dilution method. A series of two-fold dilution of each extract ranging from 500 to 0.9 mg/mL was made in the Mueller–Hinton broth. Total 100 µL of standard inoculum of the bacterial strain matched to 0.5 McFarland's standard (1.5 × 108 CFU/mL) was seeded into each dilution. The extract and growth media alone represented the positive control, and the extract with bacteria and growth media only represented the negative control. All test tubes were incubated at 37°C in the presence of 5% CO_2 for 24 hours. The tubes were checked for turbidity after incubation for a day, which denotes bacterial growth. Minimum inhibitory concentration was determined as the highest dilution of the extract that showed no turbidity.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by selecting the tubes that showed no bacterial growth during the MIC determination. Total 10 µL from each tube was subcultured on Mueller–Hinton agar plates and further incubated at 37°C for 24 hours. After incubation, MBC was defined as the lowest concentration at which no bacterial growth (no colonies) was observed on the agar plate.

Collection of Salivary Sample

The high caries risk patients were selected among the patients who reported to the Department of Conservative Dentistry and Endodontics, SRM Dental College, Chennai, Tamil Nadu, India. All the patients were instructed to brush both morning and night with nonfluoridated toothpaste prior to saliva collection for 7 days. Patients were asked to expectorate unstimulated saliva after which it was stored in dry plastic vials. The collected saliva was used within 1 hour of collection. The collected salivary sample was used as inoculum to cultivate mixed bacteria and *S. mutans* selectively. The salivary bacteria were grown in tryptone soy broth at 37°C and OD_{600} adjusted to 0.8×10^8 CFU mL^{-1}.

Grouping of the Samples

The mouthrinses were grouped as follows: group I—10% aqueous extract of *M. pudica* L. mouthrinse, group II—0.2% CHX mouthrinse, and group III—saline.

Immediate Effect on Salivary Bacterial Metabolism

The effect of test rinses on the redox activity of bacteria was assessed using resazurin-based dye, microbroth assay utilizing the methodology followed by Shiloh et al.[10] Following incubation at room temperature for 10 minutes, cultures of salivary bacteria were examined for viability. Then the samples were exposed to the test organisms for 10 seconds and immediately added with 6% resazurin dye [alamarBlue™ viability indicator (Life Technologies, Paisley, UK)]. In metabolically active cells, this blue, nonfluorescent dye can be reduced to pink allowing for a quantitative measurement of cell viability. After 10 minutes of incubation at room temperature, the culture was examined under spectrophotometer at 570 nm to assess the bacterial viability.

Antibiofilm Efficacy

Preparation of Enamel Slabs

Fifteen enamel slabs were prepared from sound enamel of human third molar teeth, extracted for surgical purposes. Dimensions of approximately 5 × 4 × 4 mm enamel slabs were prepared from facial and lingual surfaces using a low-speed diamond disk (IsoMet, Buehler, USA) under running tap water as a coolant. The convex enamel surfaces were flattened about 0.5 mm using 800-grit silicon carbide paper under water coolant, and this flat enamel surface was then polished with water-based diamond paste (0.25-µm diamond particles). Finally, the enamel slabs were ultrasonically cleaned with Milli-Q water for 15 minutes followed by sterilization by autoclaving.

Antibiofilm Evaluation on Enamel Surface Using CLSM

Enamel slabs were positioned vertically into 24-well tissue culture plates for bacterial attachment. Wilkins–Chalgren broth was dispensed into it slowly. The broth was then inoculated with sterile artificial saliva (0.5 mL) and 10 µL of *S. mutans* (cultured from human saliva) previously grown and incubated anaerobically for 24 hours at 37°C for biofilm formation. After an initial 24 hours of incubation, the medium was refreshed with sterile medium twice a day. After 7 days, the enamel slabs were removed from the anaerobic work station and treated by transferring them to a new culture plate containing the respective mouthrinses ($n = 5$) for 2 minutes at room temperature. Then the enamel slabs were gently dipped in 1 mL of phosphate-buffered solution to remove excess treatment and planktonic bacteria. The slabs were then placed on glass slides, with biofilm facing upwards. For viable staining, combination of fluorescent dyes, that is, propidium iodide and SYTO9 was applied directly to the biofilm and covered with a glass coverslip. Slides were left to stand in the dark at room temperature for 15 minutes to allow the stains to develop. Biofilms were visualized and evaluated by CLSM (Carl Zeiss,

Germany) at 40× magnification and qualitatively analyzed for the presence of fluorescence either viable (green) or dead (red) bacteria. The percentage of viability was determined using Image J software.

The Statistical Package for Social Science (SPSS) version 10.5 software was used for statistical analysis. Mean values were estimated and analyzed using one-way ANOVA test and *p*-value of <0.05 was considered as statistically significant.

Results

MIC and MBC

Zero turbidity was seen in dilutions 1:16, 1:8, 1:4, and 1:2. Thus, 1:16 dilution was confirmed to be as MIC of *M. pudica* L. leaf extract. Minimum growth of 1–5 colonies was seen in dilutions 1:4 and 1:2. Thus, 1:4 dilutions were confirmed to be MBC of *M. pudica* L. leaf extract.

Immediate Effect on Bacterial Metabolism

Table 1 shows the OD values of each group obtained by mean viability indicated by redox indicator dye by bacterial metabolism. All the groups showed a significant difference between them after 10 seconds exposure. Based on OD values, CHX (group II) showed higher antibacterial efficacy with significant difference with groups I and III as shown in Table 1.

Antibiofilm Evaluation

The treatment with CHX and *M. pudica* L. resulted in large differences in biofilm density as compared to the control as seen in Figures 1A to C. The live/dead assay revealed that there was statistically significant difference among all the groups. The density of dead bacteria was higher in CHX group as compared to the other test groups. Chlorhexidine treated sample showed 96% dead bacteria followed by *M. pudica* L. which showed 38.42% dead bacteria while saline group showed 100% viable organisms.

Table 1: Immediate effect on bacterial metabolism—OD values were shown ($p < 0.05$)

Groups	*OD at 570 nm*
M. pudica L. (group I)	1.947 ± 0.069^{a}
CHX (group II)	1.348 ± 0.037^{b}
Saline (group III)	2.848 ± 0.053^{c}

[a,b,c]Represents statistical significant difference

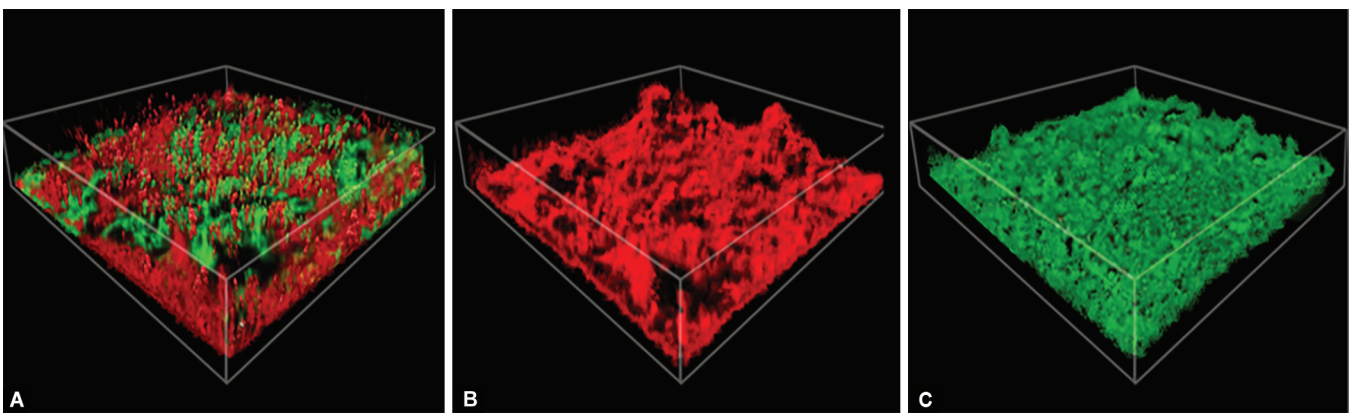
Discussion

Antimicrobial mouthrinses play a major role in reduction of oral microbial load by effectively inhibiting the bacterial growth and multiplication based on their concentration.[11] They were found to be safe and effective on topical delivery to arrest the progress of dental caries. Prepared herbal mouthwashes may provide an alternative to synthetic chemical mouthrinses with enhanced antimicrobial properties and better patient acceptance.[9] In addition, the antimicrobial activity of various herbal mouthwashes was found to be effective against cariogenic pathogens. *S. mutans* mainly metabolizes carbohydrates and synthesizes EPS which adheres tenaciously to glucan-coated surfaces with high acidogenicity and acid tolerance capacity.[12] Therefore, *S. mutans* was selected in this study for antibacterial evaluation of aqueous extract of *M. pudica* L. as a mouthrinse.

The resazurin dye had been used as an indicator of cell growth for various bacterial species.[13,14] In light of the simplicity and high-through output of the resazurin-based assay, this method was used to assess the metabolic effects of lower concentrations of test formulations on pure cultures within 10 minutes of exposure. Moreover, CLSM was used to enable the visualization of cellular viability and spatial distribution of viable biomass within intact biofilm following a single exposure. The highlights of CLSM were notable as larger biofilm clusters with bacterial inactivation depicting dead bacteria in it.[15]

The result of the study showed that CHX possesses higher antibacterial activity than *M. pudica* L. as evidenced by decreased cell viability. The antibacterial mechanism of CHX is attributed to the high affinity of positively charged CHX bisbiguanide toward negatively charged ions present in the cell membrane. The CHX molecule due to its dicationic nature, binds to the tooth surface and interacts with the bacterial membrane which leads to disorganization of the cell membrane of bacteria through the displacement of calcium ions by CHX molecules. This causes leakage of cell material and impairment of transportation of metabolites and ions through the membrane.[16] *S. mutans* biofilm was susceptible to 0.12% CHX, with a biomass reduction of over 80% observed after treatment with 0.12% CHX for 1 hour.[17] However, there is a lack of literature data to support long-term clinical evidence for caries prevention with CHX.

An exposure time of 10 seconds for typical mouthwash may reduce the microorganism count. This can be attributed to the



Figs 1A to C: Confocal scanning laser microscopy of oral biofilms following exposure to test rinses. The 3D projections show representative images of biofilm and the depth profiles plotted through the deepest section of the biofilm. Red—nonviable mass, green—viable mass. (A) *M. pudica* L.; (B) CHX; (C) saline

feature of CHX to persist in the mouth by binding to mucosal surfaces and also to the pellicle and saliva. There was no significant difference in the plaque index between patients using a 30-second rinse with a 0.12% CHX solution and those using a 60-second rinse with a 0.2% CHX solution.[18] Saliva can retain antibacterial properties and suppresses counts of bacteria in saliva for over 12 hours after rinsing with 0.2% CHX, which could be attributed to the substantivity of CHX into the mouth; however, tooth-bound CHX also accounts for the 100% reduction of bacteria.[19]

The mechanisms underlying the eradication of oral biofilm upon exposure to CHX are related to ionic interactions between the positively charged CHX molecules and negatively charged EPS matrix. The solubility, hydrophobicity, and localized charge along the EPS chain can affect its structure and the degree of its bonding with adjacent strands. The neutral net charge of the matrix reduces the repulsive forces between charged moieties reducing the volume occupied by the biofilm. The rate of biofilm also related to the various concentration of CHX as 0.2%.[20]

M. pudica L. is known for its wide range of pharmacological and biological actions due to the presence of a number of valuable bioactive compounds.[8] This herbal formulation has been proven to be biocompatible[21] and can be used to treat pain and inflammation. It has been reported to be applied locally to treat toothache. The antibacterial activity of the leaf extract may be attributed to the various phytochemical constituents present in the crude extract.[8] *M. pudica* L. has been shown to inhibit both gram-positive and gram-negative bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *and Salmonella typhi*. Phytochemical studies on *M. pudica* L. revealed the presence of alkaloids, nonprotein amino acid (mimosine), flavonoids C-glycosides, sterols, terpenoids, tannins, and fatty acids.[21] The methanolic extract of *M. pudica* L. was effective against most of the pathogens using agar well diffusion method at a concentration of 500 mg/mL. There was significant inhibition of growth with an increase in concentration.[22] However, this was the first attempt to check its action against *S. mutans* cultured from saliva of high caries risk patients.

The antibacterial action of *M. pudica* L. mainly pertains to the chemical chlorophyllin and mimosine present in the leaf extract.[23] Either individual or combined effects of the main phytochemicals such as flavonoids, saponin, alkaloids, glycosides, and tannins present in it exhibit the antimicrobial action by precipitating the microbial protein and bacterial cell lysis.[24] Erkania and Raising proved that the ethanol extract of the leaves of *M. pudica* L. responds well at a concentration of 15%, with inhibition zone diameter of 10.17 mm while at a concentration of 10%, with 8.13 mm inhibitory zone and 5% with 7.13 mm zone. The chloroform extract of *M. pudica* L. showed greater broad-spectrum activity against gram-positive bacterium *S. aureus* at the lowest concentration and methanol extract against gram-negative bacteria *Salmonella paratyphi* and *Shigella boydii* (monja).[25]

The reduced antibacterial action compared to that of CHX may be due to the lesser penetration ability of the aqueous extract of *M. pudica* L. This is also attributed to the use of lesser concentration of *M. pudica* L. Use of other methods of extract such as ethanolic and methanolic extract, application of heat to the aqueous solution, and increasing the concentration should also be considered to increase the efficacy of *M. pudica* L. in future studies.

Clinical Significance

M. pudica L. may be considered as a viable, natural antimicrobial mouthrinse for preventive strategies in medical management of dental caries. It can be formulated as an oral herbal mouthrinse or can be incorporated as antibacterial component in dentifrices to reduce oral biofilm formation in high caries risk patients.

Conclusion

Within the limitations of this study, it can be concluded that 10% aqueous extract of *M. pudica* L. was effective against *S. mutans* as a mouthrinse, but it showed less antimicrobial action than CHX. With future research on increasing its efficacy, *M. pudica* L. may be considered as a viable, natural alternative antimicrobial mouthrinse for preventive strategies in medical management of dental caries.

References

1. Fejerskov. Changing paradigms in concepts on dental caries: consequences for oral health care. Caries Res 2004;38(3):182–191. DOI: 10.1159/000077753
2. Mathur VP, Dhillon JK. Dental caries: a disease which needs attention. Indian J Pediatr 2018;85(3):202–206. DOI: 10.1007/s12098-017-2381-6
3. Aas JA, Griffen AL, Dardis SR, et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. J Clin Microbiol 2008;46(4):1407–1417. DOI: 10.1128/JCM.01410-07
4. Araújo IJ, Carvalho MS, Oliveira TR, et al. Antimicrobial activity of mouth rinses against bacteria that initially colonizes dental surface. Rev Odontol UNESP 2019;48:e20180130. DOI: 10.1590/1807-2577.13018
5. Cieplik F, Jakubovics NS, Buchalla W, et al. Resistance toward chlorhexidine in oral bacteria—is there cause for concern? Front Microbiol 2019;10:587. DOI: 10.3389/fmicb.2019.00587
6. Philip N, Leishman S, Walsh L. Potential role for natural products in dental caries control. Oral Health Prev Dent 2019;17(5):479–485. DOI: 10.3290/j.ohpd.a42741
7. Muhammad G, Hussain MA, Jantan I, et al. *Mimosa pudica* L., a high-value medicinal plant as a source of bioactives for pharmaceuticals. Compr Rev Food Sci Food Saf 2016;15(2):303–315. DOI: 10.1111/1541-4337.12184
8. Joseph B, George J, Mohan J. Pharmacology and traditional uses of Mimosa pudica. Int J Pharm Sci Drug Res 2013;5(2):41–44. https://ijpsdr.com/index.php/ijpsdr/article/view/239
9. Joshi K, Joshi AR. Ethnobotanical plants used for dental and oral healthcare in the kali gandaki and bagmati watersheds, Nepal. Ethnobotanical Leaflets 2006;2006(1):18.
10. Shiloh MU, Ruan J, Nathan C. Evaluation of bacterial survival and phagocyte function with a fluorescence-based microplate assay. Infect Immun 1997;65(8):3193–3198. DOI: 10.1128/iai.65.8.3193-3198.1997
11. Silverman S Jr, Wilder R. Antimicrobial mouthrinse as part of a comprehensive oral care regimen: safety and compliance factors. J Am Dent Assoc 2006;137 Suppl:S22–S26. DOI: 10.14219/jada.archive.2006.0406
12. Lemos JA, Palmer SR, Zeng L, et al. The biology of Streptococcus mutans. Microbiol Spectr 2019;7(1):7–10. DOI: 10.1128/microbiolspec.GPP3-0051-2018
13. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods 2007;42(4):321–324. DOI: 10.1016/j.ymeth.2007.01.006
14. Mendoza-Aguilar M, Almaguer-Villagrán L, Jiménez-Arellanes A, et al. The use of the microplate alamar blue assay (MABA) to assess the susceptibility of *Mycobacterium lepraemurium* to anti-leprosy and other drugs. J Infect Chemother 2012;18(5):652–661. DOI: 10.1007/s10156-012-0387-6

15. Reichhardt C, Parsek MR. Confocal laser scanning microscopy for analysis of *Pseudomonas aeruginosa* biofilm architecture and matrix localization. Front Microbiol 2019;10:677. DOI: 10.3389/fmicb.2019.00677
16. Brookes ZL, Bescos R, Belfield LA, et al. Current uses of chlorhexidine for management of oral disease: a narrative review. J Dent 2020;103:103497. DOI: 10.1016/j.jdent.2020.103497
17. Cousido MC, Carmona IT, García-Caballero L, et al. In vivo substantivity of 0.12% and 0.2% chlorhexidine mouth rinses on salivary bacteria. Clin Oral Investig 2010;14(4):397–402. DOI: 10.1007/s00784-009-0320-2
18. Berchier CE, Slot DE, Van der Weijden GA. The efficacy of 0.12% chlorhexidine mouthrinse compared with 0.2% on plaque accumulation and periodontal parameters: a systematic review. J Clin Periodontol 2010;37(9):829–839. DOI: 10.1111/j.1600-051X.2010.01575.x
19. García-Caballero L, Quintas V, Prada-López I, et al. Chlorhexidine substantivity on salivary flora and plaque-like biofilm: an in situ model. PLoS One 2013;8(12):e83522. DOI: 10.1371/journal.pone.0083522
20. Shen Y, Stojicic S, Haapasalo M. Antimicrobial efficacy of chlorhexidine against bacteria in biofilms at different stages of development. J Endod 2011;37(5):657–661. DOI: 10.1016/j.joen.2011.02.007
21. Seema M. Isolation, characterization and evaluation of *Mimosa pudica* seed mucilage as pharmaceutical additives. Asian J Pharm 2019;13(4):334–339. DOI: 10.22377/ajp.v13i04.3406
22. Vijaya Ramu D, Giridhar P, Ravishankar GA. Antimicrobial activity of *Mimosa pudica*. Asian J Microbiol Biotechnol Environ Sci 2004;6(4):551–552. DOI: ir.cftri.res.in/id/eprint/10135
23. Banu N, Pavithra S. A review on the bioactivity of various metallochlorophyllin—a chlorophyll derivative. Res J Pharm Technol 2017;10(2):480–486. DOI: 10.5958/0974-360X.2017.00096.8
24. Tungmunnithum D, Thongboonyou A, Pholboon A, et al. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. Medicines (Basel) 2018;5(3):93. DOI: 10.3390/medicines5030093
25. Erikania S, Raising R. Comparison of tooth paste gel antibacterial activity of ethanol extract and ethyl acetate Mimosa pudica folium against bacteria Streptococcus mutans caries dental disease. Aloha Int J Health Adv 2019;2(7):153–157. DOI: 10.33846/aijha20701