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Evaluation of *Mangifera indica*, *Anacardium occidentale leaf extracts* and 0.2% Chlorhexidine gluconate on disinfection of maxillofacial silicone material surface contaminated with microorganisms - An invitro study

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ABSTRACT

Maintenance of the quality and hygiene of maxillofacial prosthesis allows to maintain the health of the residual tissues. Sampling of the maxillofacial prostheses has relieved presence of microbial colonization on silicone surfaces. Cleaning procedures of maxillofacial silicones are done using mechanical means or using adjunctive with chemical means. Cleaning with a 2–4% chlorhexidine gluconate spray or dipping in solution for a minute and then washing under running water can sufficiently condition to reduce the amount of bacterial contamination. Due to rising microorganism resistance and fewer adverse effects, phytoextracts appear to be a viable option. Additionally, the use of excipients derived from plants is provides new opportunities for the pharmaceutical industry into the creation of innovative pharmaceutical products that are sustainable.

Aim: To evaluate and compare the leaf extracts of *Mangifera indica (M.indica), Anacardium occidentale (A.occidentale)* and 0.2% chlorhexidine gluconate (CHX) on disinfection of maxillofacial silicone material surface contaminated with *Staphylococcus aureus (S.aureus) and Candida albicans* (*C.albicans)*.

Methods: Of the 150 maxillofacial silicone elastomer silicone samples, 75 samples were contaminated with *S. aureus* and 75 with *C.albicans*. The contaminated disc was rolled on blood agar and pre-disinfection Colony Forming Units (CFU) were evaluated followed by subjecting the discs to disinfection protocols. The contaminated discs with *S. aureus* and *C.albicans* were disinfected using *M.indica* leaf extracts, *A.occidentale* leaf extracts and 0.2% CHX for 10 min. Post-disinfection CFUs were evaluated by rolling the disc on blood agar. The results were tabulated and analysed using dependent *t*-test, one-way ANOVA and Tukeys multiple posthoc procedure.

Results: Pair-wise comparison of pre-and post-disinfection log CFU counts of *S.aureus* gave a statistical significance between 0.2% CHX and *and M.indica* leaf extract. No statistically significant results were found between 0.2% CHX and *A.occidentale*. Pair wise comparison of the log CFU from pre-disinfection to post-disinfection of *C. albicans* gave a statistical significance between all the three groups.

Conclusions: In the present study *A.occidentale* leaf extract *and M.indica* leaf extract have shown significant reduction in CFU of both the organisms. 0.2% CHX showed the most CFU reduction post disinfection of maxillofacial silicone material surface contaminated *S.aureus and C.albicans* followed by *A.occidentale* leaf extracts and *M.indica* leaf extracts. Given the limitations of the current research, *A.occidentale* leaf extract *and M. indica* leaf extract can be used as an alternative for disinfection of maxillofacial silicone prosthesis.

1. Introduction

Maxillofacial prosthetics is the science and art of reconstructing anatomically flawed or missing parts of the head and neck to improve their function and appearance.[1] The use of maxillofacial prostheses provides a pleasing appearance and allows patients to resume daily routine in society.[3] For fabricating maxillofacial prostheses, the introduction of room-temperature vulcanizing polymers (e.g., MDX-4-4210;

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VST-50) has been an improvement over polymethyl methacrylate, polyvinyl chloride, and polyurethane.[3]Silicone elastomers a due to its adaptability, patient comfort, skin-like smoothness, and capacity for both intrinsic and extrinsic colour matching, silicone is the material of choice.[2–6]A delicate synergy prevails between the skin's bacterial ecology and the host. This balance may be altered when a silicone elastomeric prosthesis is placed on the skin. The compression, heating, humidity, and skin secretions and contact from the prosthesis could induce dermatitis and also periimplantitis.[4] Problems associated with the microflora of these prostheses include endophthalmitis, bacterial dermatitis, disagreeable odours, and black patches on the prosthesis.[4–9]

The skin and surfaces of the prosthesis have been routinely found to be colonized with *Staphylococcus aureus (S.aureus)*. It is also a pathogenic bacterium that has been used to examine the effectiveness of antibiotics.The presence of bacteria and yeast is observed after sampling the maxillofacial prosthetics' surface. The most common bacterial species were *Staphylococcus epidermidis, Staphylococcus schleiferi, Staphylococcus xylosus,* and *Staphylococcus capitis*, whereas the most common yeast species were *Candida albicans (C. albicans), Candida parapsilosis,* and *Candida famata*.[5] Colonization of *C. albicans* on silicone surfaces has been associated with the staining of maxillofacial prostheses.

Under all of the current cleaning techniques, patients are obliged to clean their prostheses. Some cleaning methods include wiping down with a cotton ball immersed in a moderate soapy fluid, using a brush with soap, washing in water, patting dry with a napkin, and keeping in a container out of direct sunlight. Some of the most often used cleansing agents for the facial silicone elastomer include neutral soap; peroxides, acid enzymes, sodium hypochlorite, cleansing tablets, and chlorhexidine gluconate (CHX).[1,2,7,10]

More study is required to seek different disinfection methods that do not affect the silicone surface and are both safe and non-toxic.[1] One of the key strategies for overcoming these challenges is the use of phytoextracts, which appear to be a viable disinfectant due to increased microorganism resistance and fewer side effects.

Phytochemical analysis of *Mangifera indica (M.indica)* leaf extracts indicated that there was active pharmacological components which include tannins, saponins, flavonoids, alkaloids, and mangiferin.[11–13] Because of its antioxidant, antibacterial, and anticancer properties, *Anacardium occidentale* (*A.occidentale)* plants are used medicinally in many nations.[13-16]Thus, this research was performed with the aim is to evaluate leaf extracts from *M.indica* and *A.occidentale* and to test their effectiveness against the growth of *C. albicans* and *S. aureus*, two microorganisms commonly found in the maxillofacial prosthesis, and it was compared with 0.2% CHX.

2. Material and methods

2.1. Ethics statement

The Institutional Ethical Committee approval was obtained (certificate number: 1456).

2.2. Fabrication of maxillofacial silicone material disks

Disc-shaped samples were fabricated using a metal mould of diameter 5 mm and thickness-2mm^2 using maxillofacial silicone elastomer (Silastic MDX4-4210, BioMedical Grade Elastomer Dow Corning Corp, USA). In the Vacuum mixing machine **(**Easymix Bego Wilhelm – Herbst -str) silicone catalyst was combined with base paste in accordance with manufacturer recommendations. Following its preparation, the silicone was inserted into the mould matrix and its surface was flattened with a spatula at the matrix's edge to a thickness of 2 mm. To finish the polymerization process, the matrix containing the silicone samples was left in the mould for three days with the exterior surface exposed to the room environment. Then the specimens were taken out of the mould using a fine-pointed tool, and extra material and imperfections were trimmed out using thin, curved scissors.[2] The study excluded those specimens with visual surface defects, deformities and gross irregularities and specimens with inaccurate dimensions.

2.3. Extract preparation

Fresh leaves were collected from an infestation-free *M. indica* and *A. occidentale* tree in the region of Thivim, Goa, India (5° 35′ 59.99″ N, 73° 47′ 59.99″ E).The leaves were authenticated by the Central research facility of Shri BMK Ayurveda Mahavidyalaya, Belagavi. The leaves were shade dried for a period of 15 days and then made into a medium-coarse powder by using a grinder and stored in an airtight container until extract is made. A weighed quantity of the leaf powders was extracted with 99% ethyl alcohol (Ethanol, absolute, Changshu Hongsheng Fine Chemical Co., Ltd, Jiangsu Province) as solvent by Soxhlet extraction method.[17] The extracts were filtered and concentrated using a water bath till all the solvent evaporates. The extracts were stored in a refrigerator until further use.

2.4. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal concentration (MBC/MFC) of M.indica and A.occidentale leaf extracts against S. aureus and C. albicans and disinfectant preparation

By using the broth dilution procedure, the MIC of the leaf extracts was determined Suspension of *S. aureus* and *C. albicans* were added to Brain Heart Infusion (BHI) broth to which graded amount of freshly prepared leaf extracts were added. Series of dilution were prepared containing same volume of media inoculated. One test tube was left without extract, to serve as positive control and one without organism to serve negative control. Final volume per tube is 1 mL which includes 10 μL of the organism in each tube and different concentrations of extract and the BHI broth. The solutions will be incubated at 37° for 24 h. MIC was taken as the least concentration of extracts that showed no observable growth. Samples from the tube that showed no visible bacterial growth during MIC determination were inoculated on separate agar plates and incubated at 37° C for 24 h. The least concentration of the extracts that showed no colonies on the medium after the incubation period was regarded as MBC/MFC. The value of MBC of *M. indica* was 75 μL against *S. aureus and C.albicans* and the value of MBC of *A. occidentale* against *S. aureus* was 25 μL and *C. albicans* was 100 μL. The disinfecting solution was prepared using the highest effective concentration of the MBC and MFC values of freshly prepared extracts. The solutions were prepared by mixing the effective concentration values of the extracts in non-ionized distilled water until a homogenous solution was obtained and stored in sterile conditions.

For contamination of maxillofacial silicone material disks with *S. aureus* and *C. albicans*, the sterilised silicone disk was placed in a test tube to which 1 mL of BHI broth and 2 μL of standardized inoculum (1×10^6 cells) of the respective micro-organism was added and incubated aerobically for 24 h at 37° C.(Fig. 1). After which, each of the disc were rolled onto blood agar in a 1cmx1cm area and blood agar plates were incubated for 24 h at 37 °C. after 24 h Colony forming units (CFU) were counted on plates for baseline that was considered the pre-disinfection CFU. The disc was placed back in the above solution and incubated for 2 h at 37 °C following which discs were subjected to disinfection.

2.5. Sample size and disinfection protocol

The sample size was calculated using the formula n = 2S2(Z1-**α**/2+Z1-**β**)2/d2 where n = 25 and the total sample size was 150, of which 75 discs were contaminated with *S. aureus* (ATCC 25923) followed by disinfection protocol of 25 silicone disc by *M.indica* leaf extract 25 by A.*occidentale* leaf extract and remaining 25 by 0.2% CHX (Hexidine, ICPA Health Products LTD) for 10 min (Fig. 2). Similarly, the other 75 discs were contaminated with *C.albicans (*ATCC 10231) and

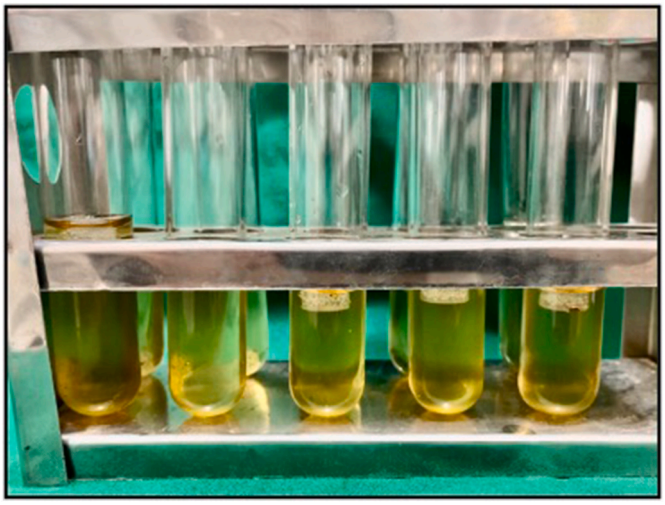

Fig. 1. Silicone disk placed in test tube containing BHI broth and organism for contamination of discs.

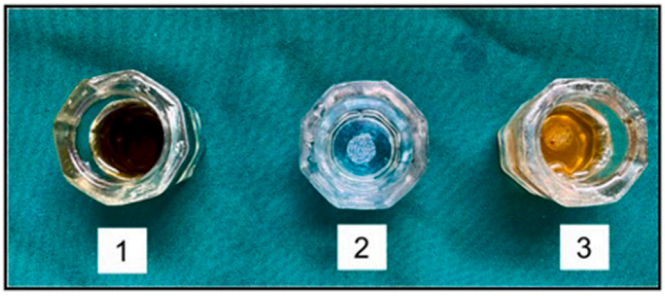

Fig. 2. Decontamination of silicone disc 1- *A. occidentale* leaf extract, 2–0.2% CHX, 3- *M. indica* leaf extract.

disinfection protocols were followed.

The blood agar was incubated for 24 h at 37 °C after which the CFU post-disinfection was counted for the respective organisms (Figs. 3 and 4).

3. Results

The colony forming units/ml of the respective microbial count was converted to log CFU and tabulated to evaluate and compare the leaf extracts of *M. indica, A.occidentale* and 0.2% CHX on disinfection of maxillofacial silicone material surface contaminated with *S. aureus* and *C. albicans.*

The results were tabulated and analysed and subjected to statistical analysis using SPSS software version 20. The mean and standard deviation of the pre-disinfection of *S. aureus* was 8.12(0.008), 8.13(0.008), 8.13(0.008) in the 0.2% CHX*, M. indica* and *A. occidentale* groups respectively. Mean and standard deviation of the post-disinfection was 0.48(0.59), 1.95(0.73), 0.87(1.03) in the 0.2% CHX*, M. indica* and *A. occidentale* groups respectively. The dependent *t*-test was applied to evaluate pre disinfection v/s post-disinfection of *S.aureus in* each group which showed statistically significant ($p = 0.0001$) in all the groups. (Table 1).

Pair-wise comparison of 0.2% CHX*, M.indica* and *A. occidentale* with pre and post-disinfection log CFU counts of *S.aureus was done* by Tukeys multiple posthoc procedure, found that there is statistically significance between 0.2% CHX and *M. indica* ($p = 0.001$)*; M. indica and A. occidentale* ($p = 0.001$). No statistically significant were found between 0.2% CHX and *A.occidentale* ($p = 0.1988$). (Table 2).

The mean and standard deviation of the pre-disinfection of *C. albicans* was 6.02(0.06), 6.04(0.07), 6.03(0.07) in the 0.2% CHX*, M. indica* and *A. occidentale* groups respectively. Mean and standard deviation of the post-disinfection was 0.22(0.55), 1.91(0.53), 1.06(0.77) in the 0.2% CHX*, M. indica* and *A. occidentale* groups respectively. The dependent *t*-test was applied to evaluate pre disinfection v/s post-disinfection of *C. albicans* in each group which showed statistically significant ($p = 0.0001$) in all the groups. (Table 3).

Pair wise comparison of 0.2% CHX*, M. indica* and *A. occidental* of the difference of the log CFU from pre-disinfection to post disinfection of *C. albicans was done* by Tukeys multiple posthoc procedure, found that statistically significant between all the three groups; 0.2% CHX v/s *M. indica (p=0.001); M. indica v/s A. occidentale (p=0.001) and* 0.2% CHX v/s *A. occidentale* ($p = 0.001$). (Table 4).

4. Discussion

When it comes to disinfecting maxillofacial elastomer, chemical soaking is the method of choice. Cleansing with a 2–4% CHX spraying or dipping in solution for 1 min, followed by washing under running water, can adequately condition to minimize the quantity of bacterial contamination without jeopardizing the prosthesis. By rupturing cell membranes and allowing intracellular material to leak out, CHX exhibits potent antifungal and antimicrobial properties that ultimately result in cell death.[18]

The findings regarding the cleaning procedures using submersion in 0.12% and 2% CHX is shown to be successful in lowering the CFU of the

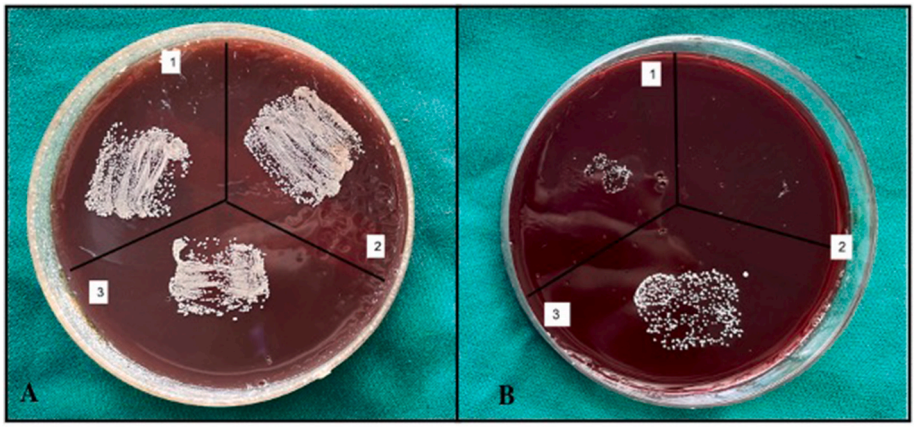

Fig. 3. A: *S. aureus* colonization on blood agar pre-disinfection. B: *S. aureus* colonization on blood agar post-disinfection. 1- *A. occidentale* leaf extract, 2–0.2% CHX, 3- *M. indica* leaf extract.

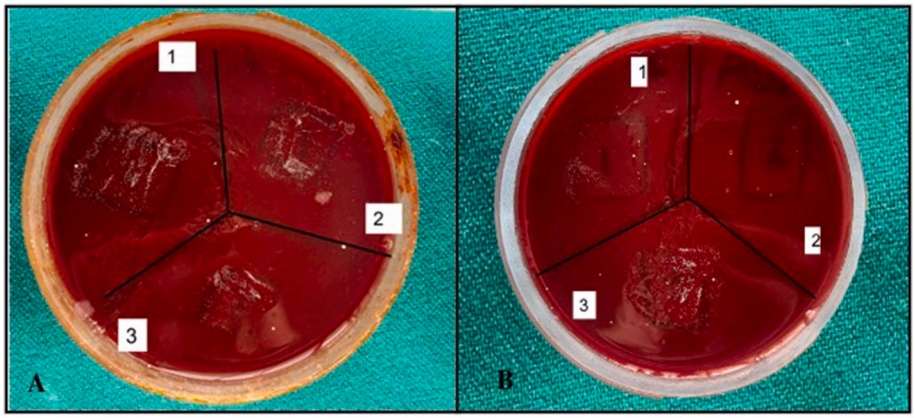

Fig. 4. A: *C. albicans* colonization on blood agar pre-disinfection B: *C. albicans* colonization on blood agar post-disinfection. 1- *A. occidentale* leaf extract, 2–0.2% CHX, 3- *M. indica* leaf extract.

Table 1
Comparison of pre-disinfection and post-disinfection log CFU counts of *S.aureus in 0.2%* CHX, *M. indica and A.occidentale* by dependent *t*-test.

Groups	0.2% CHX		*M. indica*		*A. occidentale*	
	pre	post	pre	post	pre	post
Mean	8.12	0.48	8.13	1.95	8.13	0.87
SD	0.08	0.59	0.08	0.73	0.08	1.03
Mean Diff	7.64		6.18		7.26	
SD diff	0.57		0.75		1.03	
% of change	94.13		76.01		89.29	
t value	67.34		41.3099		35.2106	
p value	**0.001***		**0.001***		**0.001***	

Table 2
Pair wise comparison of 0.2% CHX, *M. indica and A. occidentale* with pre-disinfection and post-disinfection log CFU counts of *S. aureus* by Tukeys multiple posthoc procedures.

Time points	Extracts	0.2% CHX	*M.indica*	*A.occidentale*
Pre-disinfection	Mean	8.12	8.13	8.13
	SD	0.08	0.08	0.08
	M. indica	P = 0.9503	–	
	A. occidentale	P = 0.9503	P = 1.0000	–
Post-disinfection	Mean	0.48	1.95	0.87
	SD	0.59	0.73	1.03
	M.indica	**P=0.0001***	–	
	A. occidentale	P = 0.1988	**P=0.0001***	–

*p < 0.05.

Table 3
Comparison of pre-disinfection and post-disinfection log CFU counts of *C. albicans* in 0.2% CHX, *M.indica and A. occidentale* by dependent *t*-test.

Groups	0.2% CHX		*M. indica*		*A. occidentale*	
	pre	post	pre	post	pre	post
Mean	6.0	0.22	6.04	1.91	6.003	1.0
SD	0.06	0.55	0.07	0.53	0.07	0.77
Mean Diff	5.80		4.12		4.96	
SD diff	0.56		0.51		0.76	
% of change	96.38		68.31		82.33	
t value	52.0056		40.2926		32.7426	
p value	**0.001***		**0.001***		**0.001***	

*p < 0.05.

microorganisms on maxillofacial silicones in previous studies conducted by Ariani et al.,[5] de Azevedo MN[10] and Pinheiro JB et al.[20] In contrast in the study conducted by Guiotti et al.,[2] the presence of 50% viable

Table 4
Pair wise comparison of 0.2% CHX, *M. indica and A.occidentale* with pre-disinfection and post-disinfection log CFU counts of *C. albicans* by Tukeys multiple posthoc procedures.

Time points	Groups	0.2% CHX	*M. indica*	*A.occidentale*
Pre- disinfection	Mean	6.02	6.04	6.03
	SD	0.06	0.07	0.07
	M.indica	P = 0.7293	–	
	A. occidentale	P = 0.9239	P = 0.9239	–
Post- disinfection	Mean	0.22	1.91	1.06
	SD	0.55	0.53	0.77
	M. indica	**P=0.0001***	-	
	A. occidentale	**P=0.0001***	**P=0.0001***	–

*p < 0.05.

C. albicans after 10 min of 4% CHX was observed and concluded that the most effective regimen for maintaining silicone prosthesis against *S. aureus* and *C. albicans* was hand washing with water and neutral soap.[2]

It is claimed that 4% CHX presents a surface that is altered, resulting in irregularities and ultimately microbial adhesion. Photomicrographs showed that the polymer surface was impacted by 4% CHX. Improper handling of the delicate process of maintaining the hygiene of silicone polymer maxillofacial prostheses may speed up the degradation of the material. Regular exposure to disinfection solutions can alter the properties of silicone, changing its color, hardness, and tear resistance.[18] A study conducted by Chotprasert N et al. found that after simulating once-daily disinfection for a year, silicone disinfected with a 2% chlorhexidine solution and liquid soap showed the highest color change.[19] In the present study a lower concentration of 0.2% CHX was selected for the comparison and control purpose as it is the most common, economical and easily available in Indian markets.

The use of phytoextracts appears to be a viable disinfectant due to increased microorganism resistance and fewer side effects. In the current research, taking this into account in the comparison was done between 0.2% CHX, *M.indica and A.occidentale* leave extracts disinfectant solution. The disinfectant solution was formulated and prepared for the purpose of this study and mango and cashew were chosen as the plant extracts for their established antifungal and antimicrobial properties in previous studies.[11–16] The study's rationale and strength is derived from the selection of these particular plants are factors such as the availability of these plants in the local region, their historical use in traditional medicine, and the presence of bioactive compounds that could aid in disinfection. Moreover, using locally available plants aligns with the idea of sustainable and culturally relevant solutions for healthcare applications.[21]

Numerous research studies have linked the antimicrobial qualities of *M. indica* to the presence of phytochemicals like phenolics, tannins,

alkaloids, flavonoids, and the unique mango xanthonoid known as mangiferin. It has been demonstrated that *M. indica* leaves possess antimicrobial qualities, such as antibacterial, antiviral, antifungal, and anti-plasmodial effects.[21] Galactotannin is a phytochemical that has a strong affinity for iron and attaches itself to the metal on the surface of microorganisms to form a complex.It has been discovered that this action operates in two stages. It attaches itself to microbial protein first. Second, it makes the microbial proteins aggregate, which leads to precipitation. Microbial cell membrane fluidity and other microbial membrane properties are altered as a result of these effects. Their antioxidant properties and ability to scavenge free radicals and reactive oxygen species are well known. Their permeability to microbial cell membranes increases their pharmacological activity.[21]

Significant antibacterial and antifungal activity were discovered in an ethanol extract of *A occidentale* leaves.[13,22] Plant metabolites such as phenols, flavonoids, tannins, anthocyanin and Carotenoids are found in leaf extracts and are responsible for their antibacterial properties. High levels of tannins in cashews interact and precipitate proteins thereby inhibiting the growth of pathogens.[13–16]

This prompts further probing and research into various therapeutic and pharmacologic applications of *M. indica* (mango) and *A. occidentale* (cashew) leave extracts to utilize as disinfecting solutions. In comparison to the water-based extracts, the leaf alcoholic extracts showed more anti-bacterial and anti-fungal activity; this might be because alcoholic extract can better extract the bioactive agents and producing more antimicrobial properties.[14] Hence ethanolic extracts *of M. indica and A. occidentale* were used. Bhat et al.[23] developed a mouthwash using extract from mango leaves and assessed its antimicrobial properties, as well as its anti-gingival inflammation and anti-plaque accumulation properties. The results of the study showed that the growth of the microbial population in the test samples was inhibited by *M. indica* leaf mouthwash (2%) Additionally noted were improvements in gingival health and a decrease in plaque accumulation.

In the present study, *A. occidentale* leaf extract disinfectant performed statistically better than *M. indica* leaf extracts among the two extracts for both *S aureus* and *C. albicans.* In a study conducted by Anand G et al.[13] there was no difference in the antimicrobial action of *A. occidentale* leaf extract against *S. aureus, C. albicans* compared to CHX-based mouth rinse.[13] Other studies have stated that used plant-based extract solutions, such as *C. nardus* (citronella),[2] alcoholic solution of 10% green propolis,[10] 10% Ricinus communis[18] solution were effective in eliminating *S. aureus* biofilms from maxillofacial elastomers.0.2% CHX had a superior disinfecting action on *S. aureus* and *C. albicans* followed by *A. occidentale and M. indica*. The action of bioactive component of *M. indica and A. occidentale* gave statistically significant and overall acceptable action against biofilm formation of *S. aureus and C. albicans.* Hence the use of these natural disinfectants can be a reliable alternative for maintenance of not only maxillofacial prosthesis but even denture hygiene.

The study has some limitations, such as the fact that the time period considered for disinfection may be different from that determined for individuals who have maxillofacial prostheses of various types and sizes. Leaves from different geographical areas, in different seasons, can yield a different result. Since this is an in-vitro study, the application of the results in clinical conditions might yield a different result.

Additional studies can be conducted to gauge the impact of the disinfectant solutions on the colour stability, shore A hardness, tear strength, and surface roughness of silicone materials. Further research can be suggested to assess the combined effect of *M. indica* leaf and *A. occidentale* leaf extracts on the micro-organisms commonly seen in the oral cavity. A combination of mechanical methods of brushing and immersion in disinfectant solutions can be studied.

5. Conclusion

Given the limitations of the current research, the following conclusions can be made. *A. occidentale* leaf extract and *M. indica* leaf extract when used for the disinfection of maxillofacial silicone material surface *contaminated with S.aureus* and *C.albicans* resulted in a significant reduction in post-disinfection CFU.

0.2% CHX showed the most CFU reduction post disinfection of maxillofacial silicone material surface contaminated *S. aureus and C. albicans* followed by *A. occidentale leaf extract and M.indica* leaf extract. *A.occidentale* leaf extract *and M.indica* leaf extract can be used as an alternative for disinfection of maxillofacial silicone prosthesis.

Consent

The study being an in vitro investigation did not involve human subjects or patients directly.

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Declaration of competing interest

No potential conflict of interest relevant to this article was reported.

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