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Research Article

Comparative Evaluation of Denture Disinfectant Potential of White Vinegar, Copper Water and Chlorhexidine – an *Invitro* Study

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Abstract

Objectives: To compare and evaluate the antimicrobial effect of White vinegar, Copper water and chlorhexidine when used as immersion disinfectants for PMMA resin.

Materials and Methods: One hundred and sixty acrylic denture base resin specimens of dimensions 10 x 10 x 2 mm were made. Four groups of cleansing treatments were carried out. Group 1 - Sterilized distilled water, Group 2 - White vinegar diluted with water (1:1), Group 3 - Chlorhexidine mouth wash (2%), Group 4 – Copper water (sterile water stored in copper vessel for 24 hours). Candida albicans and Staphylococcus aureus were used as reference organisms and used for inoculating the specimens. Viability of organisms were assessed after immersing in the disinfectant media. Colony forming units were counted at the end of I hour and 6 hours. The results were statistically analyzed using one way ANOVA and Tukey's Post hoc multiple comparisons.

Results: CFU/mL values of S. aureus obtained at the end of one hour were as follows: Gr 1- 1.20 x 104, Gr 2- 0.21 x 104, Gr 3-0.57 x 104 and Gr 4-1.05x104. At the end of six hours the values were 2.46 x 105, 0.20 x 104, 0.37 x 104 and 0.19 x 104 respectively.

CFU/mL values of C. albicans obtained at the end of one hour were as follows: Gr. 1-8.43 x 104, Gr 2-0.62x104, Gr 3-1.39 x 104and Gr 4-0.08 x 104. At the end of six hours the values were 4.60 x 105, 0.13 x 104, 0.08 x 104and 0.27 x 104 respectively.

Conclusions

- Copper water which is made by storing water in copper vessel for at least six hours can be used as an immersion disinfectant medium for acrylic prostheses.
- White vinegar is a potent disinfectant medium which can be used as an overnight storage medium for dentures.
- The present study endorses the already established fact that Chlorhexidine gluconate has antimicrobial effect which can be used as an overnight storage medium for dentures.
- All the three media mentioned above have antimicrobial effect against Candida albicans and Staphylococcus aureus which were used as reference organisms.

Keywords: Denture Cleanser; Vinegar; Copper Water; Candida Albicans; Staphylococcus Aureus; Disinfectant Immersion Medium

Introduction

In a demographic survey conducted by the United Nations Population fund, on the aging population of India, it was observed that by 2050, twenty percent of the population will be constituted by aged individuals above 60 years. 16% of the aged individuals is estimated to be completely edentulous. As a sequel, the number of denture wearers will be increased and who will be solely dependent on dentures for mastication. Oral cavity is vulnerable to microbial colonisation because of the morphological characteristics that favour plaque accumulation. Wearing of acrylic dentures can aggravate the conditions of microbial colonisation. It has been observed that dentures harbour different pathogenic bacteria capable of causing local and systemic diseases [1-4].

Removable dentures in use are never considered as sterile. When a prosthesis is inserted, within a few hours the interface will be colonized by the oral biofilm and subsequently by microorganisms. Denture stomatitis is commonly found in fifty percent of the denture wearers [5,6]. Candida is primarily responsible for the causation of stomatitis. There is an evident increase in the oral candida levels along with the longevity of the dentures. Older the denture, higher will be the candida levels. In an attempt to control the candida, old denture is replaced with a new denture and the candida levels suddenly drops but within six months the levels get restored [7]. The organisms that colonise the fitting surface of the denture are usually facultative anaerobes. Commonly studied microorganism of the fitting surface of the denture is Candida albicans because of the association with denture stomatitis. When a person wears dentures for one year, the following species of microorganisms usually colonise the mouth viz. Streptococcus (11%), Staphylococcus aureus (10%), Candida Albicans (5%), K. Pneumonia (4%), Diphtheroid (5%), Lactobacillus (1%) and many other organisms with minor presence [4,8,9].

Maintenance of hygiene is of critical importance to denture wearers. Use of soap, dentifrices, denture brush, oxygen releasing tablets that release oxygen, sodium hypochlorite and Chlorhexidine are commonly recommended for the cleansing of dentures. Many patients are not convinced of the effectiveness of these products and many cannot afford. Simply keeping the dentures immersed in water overnight cannot ensure adequate hygiene standards; in fact, without employing proper cleansing methods, if the dentures are kept immersed in water, it can be detrimental. During the water storage, the candida can grow further in unimaginable proportions [10,11]. Denture stomatitis can be controlled effectively by employing short term disinfection protocols. Chlorhexidine gluconate and Sodium hypochlorite are used in the treatment of denture stomatitis. Though economical and effective in disinfection, the corrosive nature of sodium hypochlorite is a deterrent and at times it causes irritation to both skin and mucosa. Both patients and dentists favour the use of chlorhexidine [12,13]. Another reason for the acceptability of Chlorhexidine gluconate is because of its adaptability that it can be used both as a mouth wash and as a disinfectant denture immersion medium. It can limit the adhesion of microbes to oral mucosa as well as the denture and minimizes the biofilm formation. As a mouth wash, it is used at 0.2% concentration and as an immersion disinfectant 2% concentration is made use of [14,15].

Antimicrobial property of copper was found out by Egyptians as early as 2600 BC. Large deposits of copper were found in Cyprus from which the Latin name of copper – Cuprum – was derived. In ancient times copper was used to treat infections and wounds. Drinking copper water (water stored in copper vessels) has been recommended by Ayurvedic physicians for promoting blood circulation, curing digestive problems, healing of wounds and for boosting hemoglobin. The workers of copper industry were not affected by the Cholera epidemics of the 19th century probably because of the presence of copper in their system. Many dental alloys including amalgam contains copper which might have resisted to some extent secondary caries in restored teeth. Nano particles of copper (1-100nm) have been proven to inhibit growth of microorganisms like streptococcus mutans, staphylococcus aureus and candida albicans [16-20]. The antimicrobial property of copper is related to the damage caused to cell functions of the microbes. Copper can adhere to the metalloproteins of the cell wall and can produce reactive oxygen species (ROS) and eventually it damages the cell walls. Methicillin resistant staphylococcus aureus (MRSA) has been effectively controlled by copper. A few studies have been conducted on the effectiveness of copper on fungi. The mechanism of action is similar both on bacteria and fungi in causing cell membrane damage. [21-25] Antimicrobial potential of copper water is proven but it has never been employed in disinfecting the dentures or in treating denture stomatitis.

In our country, denture cleansing along with disinfection is done with proprietary pharmaceutical products like chlorhexidine gluconate and house hold products like vinegar. Copper water which is obtained by storing water in a copper vessel for a specified time can be included in this class of materials that are used for denture cleansing but it needs scientific validation.

In the context described, the present invitro study was designed with the following objectives:

-to compare the antimicrobial effect of 1. Chlorhexidine gluconate (2%), 1. White vinegar (50%) 2. Copper water (made by storing distilled water in a copper vessel for 24 hours) and Chlorhexidine gluconate (2%)

-to compare the effectiveness of the above three materials when used as immersion media to disinfect heat cure acrylic denture base resins kept for one hour and six hours.

Methodology

The present study was conducted to compare and to evaluate the antifungal and antibacterial effect of White vinegar, Copper water (distilled water stored in a copper vessel) and Chlorhexidine against PMMA specimens inoculated with Staphylococcus aureus and Candida albicans. Plane distilled water was used as the control medium.

Fabrication of heat polymerized acrylic specimens

One hundred and sixty specimens measuring 10x10x2mm were made in heat cure acrylic resin by duplicating similar sized metallic dies. Two piece moulds were prepared using elastomeric impression material (silicone putty) and dental stone in a denture flask. Acrylic dough was packed in the putty side of the mould and processed according to the manufacturer's instructions (Figure 1,2). The specimens were then retrieved, ultrasonically cleaned and immersed in distilled water at 37ºC for 48 hours to eliminate the residual monomer.

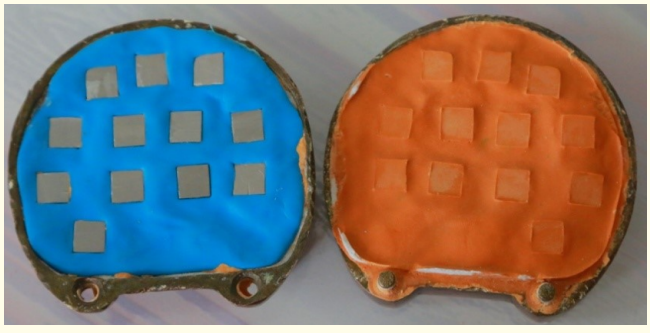

Figure 1: Mould preparation.

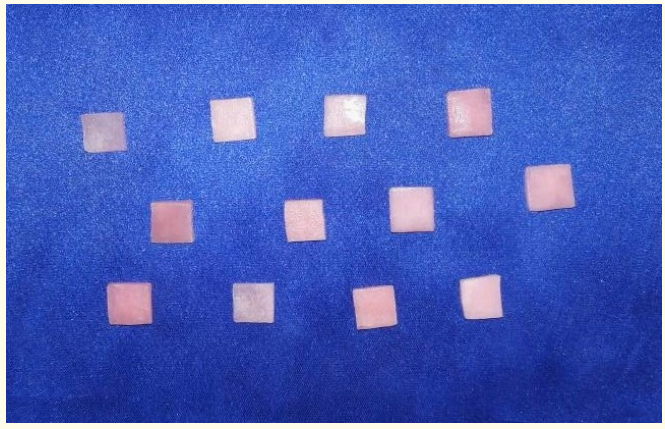

Figure 2: PMMA specimens.

Microorganisms

Two strains of microorganisms viz. Candida albicans and Staphylococcus aureus were selected as reference organisms for the experiments.

Microbial inoculation of specimens

Candida albicans was separated from the clinical environment, cultured in Sabouraud dextrose agar (SDA), and incubated at 37°C for 48 hours. After incubation, cells were harvested and adjusted to make a suspension of 10^7 CFU/mL and used as the fungal solution for inoculating the acrylic specimens. Staphylococcus Aureus was resuscitated and cultured in agar medium until it reached the logarithmic growth phase. The bacterial solution was made and diluted with Tryptic soy broth (TSB) medium to get final concentration of $1x10^6$ CFU/mL and used for inoculation of the specimens.

Before inoculation, the specimens were autoclaved under 15 pounds pressure at 121˚C, for 15minutes. The specimens were grouped into two consisting of eighty in each. One group of specimens was inoculated with Candida albicans and the other group with Staphylococcus aureus. SDA plates were prepared and swabbed with 20µL of test organisms. Three specimens were placed on each plate. The plates were incubated at a temperature of 37˚C for 24 hours. After incubation, the specimens were removed with sterile forceps and washed with 5ml of distilled water. The number of colonies forming units of Candida albicans and Staphylococcus aureus were counted with a bacterial colony counter (Figure 3).

Figure 3: CFU counter.

The specimens inoculated with Candida Albicans and Staphylococcus Aureus were further divided into four groups. The distribution of groups is given in table 1. Group 1 was considered as control and was immersed in distilled water. Groups 2, 3 and 4 were the experimental groups and were taken for immersion in different media to test the antimicrobial potential.

The immersion media used for experimental groups

Group- 1 specimens were immersed in distilled water kept in glass test tubes. Group- 2 specimens were immersed in White vinegar (50%) prepared by diluting with distilled water in 1:1 proportion by volume. Group 3 specimens were immersed in Chlorhexidine (2%) further diluted by distilled water in 1:1 proportion. Group 4 specimens were immersed in copper water (distilled water stored in copper vessel for 24 hours) (Figure 4-9).

Type of specimens (160)	Gr.1. Control-Inoculated specimens immersed in distilled water	Gr.2.Experimental-Inoculated specimens immersed in White Vinegar (50%)- diluted with distilled water – 1:1	Gr.3.Experimental-Inoculated specimens immersed in Chlorhexidine (2%)- diluted with distilled water – 1:1	Gr.4.Experimental-Inoculated specimens immersed in distilled water stored in Copper vessel for 24 hours
Specimens inoculated with Candida Albicans (80)	20 specimens	20 specimens	20 specimens	20 specimens
Specimens inoculated with Staphylococcus Aureus (80)	20 specimens	20 specimens	20 specimens	20 specimens

Table 1: Distribution of specimens.

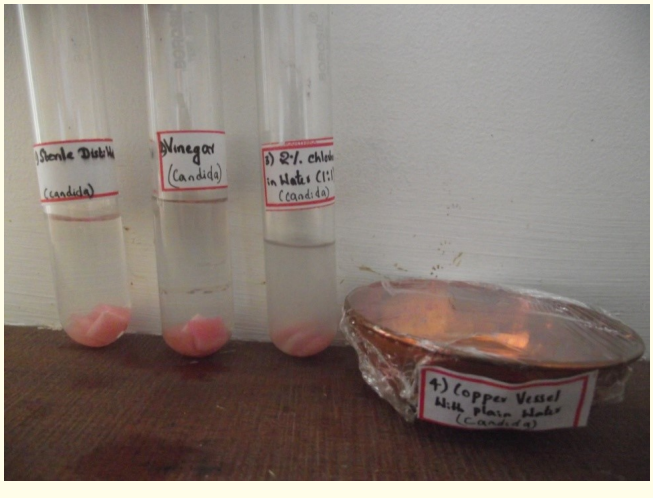

Figure 4: Candida inoculated specimens were immersed in different media.

Figure 5: Staphylococcus aureus Aureus inoculated specimens immersed in different media (Gr 1-4).

Figure 6: Inoculated specimens were placed in Phosphate Buffered Saline solution

Figure 7: White vineger used for immersing the specimens to test antimicrobial potential.

Figure 8: Chlorhexidine used for immersion of specimens to test the antimicrobial potential.

Figure 9: Copper vessel used to store distilled water and for immersing specimens to test the antimicrobial potential.

Group 1 to 4 consisting of 20 specimens in each, of which 10 specimens were taken from each after one hour and were suspended in 1ml sterile Phosphate Buffered Saline and which was mixed well (Figure 6). 10µl from each sample was plated (spreading microbial culture on to solidified nutrient media) and incubated at 37^0C for 24 hours. Specimen without any treatment was also plated. Then the plates were observed for the presence or absence of colony forming units (CFUs). The CFUs were counted using a bacterial colony counter and the values were expressed in CFUs/mL. Same protocol was repeated after six hours using the remaining 10 specimens from each group (Figure 10, 11).

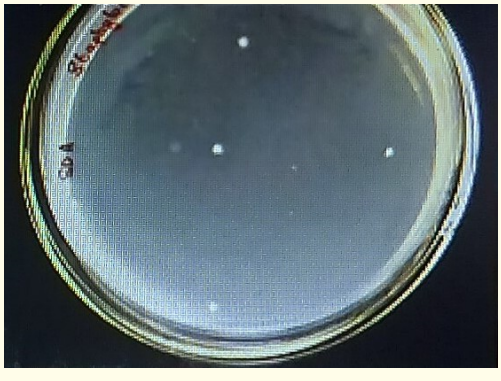

Figure 10: Few colonies formed in the plate.

The values were tabulated and were statistically analyzed using one way ANOVA and Tukey's Post hoc multiple comparison test. Summary of the methodology is given in the flow chart (Figure 12).

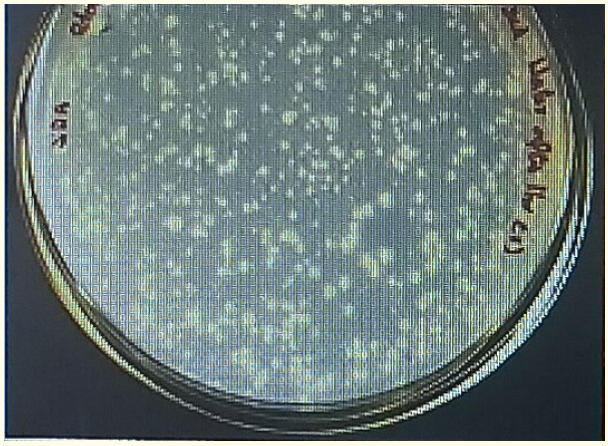

Figure 11: Colonies formed in abundance in the plate.

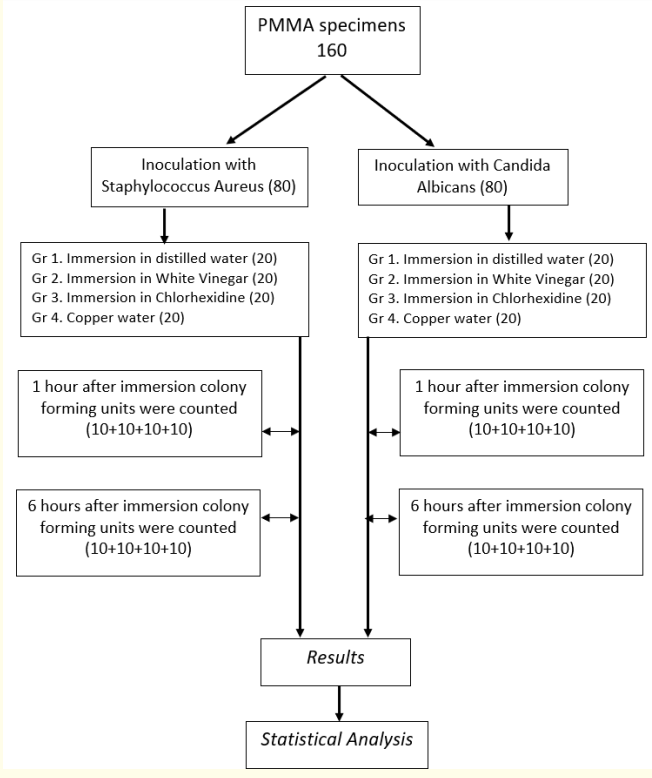

Figure 12: Flow chart on methodology.

Results

The antifungal and antibacterial effects of denture immersion media containing (i)White vinegar (50%), (ii)Chlorhexidine (2%) and (iii) copper water (distilled water kept in copper vessel for 24 hours) were evaluated. The reference microorganisms used were Candida albicans and Staphylococcus aureus which were inoculated on to the PMMA specimens. Distilled water was used as an immersion medium to serve as control. The inoculated specimens were plated and the microorganisms were allowed to grow. The specimens were then treated with immersion media. The colony forming units (CFU) were counted at the end of 1 hour and 6 hours after immersion. More number of CFUs indicated poor antimicrobial property of the immersion medium and if the CFUs were less, the antimicrobial property was considered to be high.

Statistical analysis of the data was done using SPSS statistical package (version 2.0). Null and alternate hypotheses were formulated. The p-values were calculated to find out the level of significance. If $p < 0.001$ the null hypothesis was rejected and the alternate hypothesis was accepted. If $p > 0.001$ the null hypothesis was accepted. To compare between each group (1 to 4) and the time periods (1hr and 6hrs) one way ANOVA was used. Tukey's Post hoc multiple comparison testing was done to assess the level of significance between each group.

At the end of one hour, all experimental groups showed statistically significant reduction in the number of S. Aureus colonies (Figure 13). Maximum reduction was found in Group 2 in which specimens were stored in white vinegar and the mean value was $0.21x10^4$ CFU/mL. Group 1 (control group) showed the maximum number of CFUs and the mean value was $1.20x10^4$ CFU/mL. Statistically significant reduction of colony forming units were found in all the three experimental groups ($p < 0.001$) (Table 2). The level of significance of reduction of colonies between each group was analyzed using Tukey's Post hoc multiple comparison and which showed statistically significant difference between Group 1 and 2, Group 1 and 3, Group 2 and 4. There was no significant difference between Group 2 and 3 (Table 3). For C. albicans, statistically significant reduction in number of colonies were found in all groups with $p < 0.001$ and the maximum reduction was found in Group 4 (copper water) with a mean value of $0.08x10^4$ CFU/mL. The least reduction was found with the control group without any disinfection with a mean value of $8.43x10^4$ CFU/mL (Table 4). The level of significance of reduction of colonies between each group was analyzed using Tukey's Post hoc Multiple comparison test which showed statistically significant difference between Groups 1 and 2, 1 and 3, 1 and 4, 2 and 3, 2 and 4 and 3 and 4 ($p < 0.001$) (Table 5) (Figure 15-18).

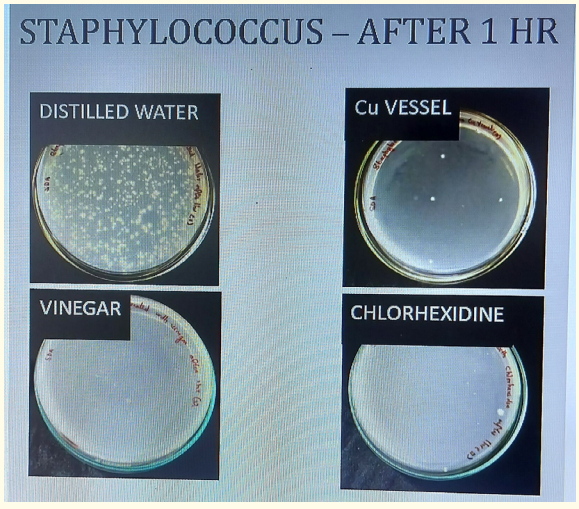

Figure 13: Staphylococcus - one hour after immersion different media.

Figure 14: Staphylococcus -6 hours after immersion in different media.

Figure 15: Candida albicans - one hour after immersion in different media.

Figure 16: Candida albicans - 6 hours after immersion different media.

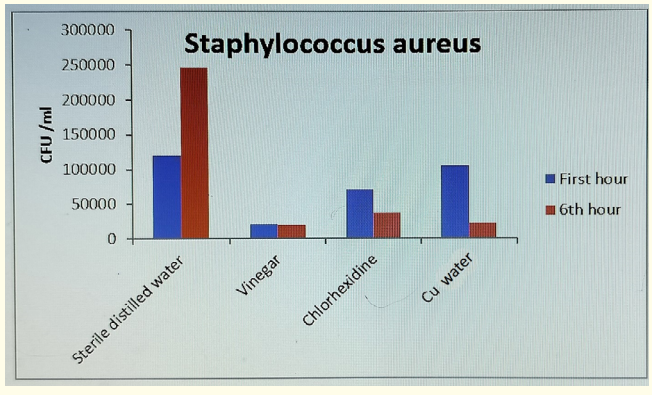

Figure 17: Comparison of CFUs of S. aureus at the end of 1 hour and 6 hours.

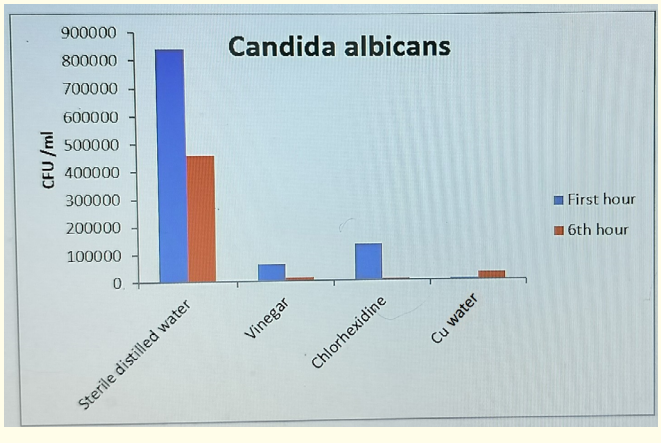

Figure 18: Comparison of CFUs at the end of 1 hour and 6 hours.

Staphylococcus aureus	N	CFU/mL		p
		Mean	SD	
Gr 1 (Distilled water)	10	1.20×10^4	0.12	<0.001
Gr 2 (Vinegar)	10	0.21×10^4	0.16	
Gr 3 (Chlorhexidine)	10	0.57×10^4	0.37	
Gr 4 (Copper water)	10	1.05×10^4	0.01	

Table 2: The mean values of colony forming units obtained for *S. aureus* at the end of one hour when different groups were compared using one way ANOVA.

Post hoc analysis		Mean Difference	Std. Error	P value
Group 1 (Distilled water)	Group 2	0.99	0.10	<0.001
	Group 3	0.63	0.10	<0.001
	Group 4	0.15	0.13	0.249
Group 2 (Vinegar)	Group 1	-0.99	0.10	<0.001
	Group 3	-0.35	0.10	>0.001
	Group 4	-0.83	0.13	<0.001
Group 3 (Chlorhexidine)	Group 1	-0.63	0.10	<0.001
	Group 2	0.35	0.10	>0.001
	Group 4	-0.48	0.13	>0.001
Group 4 (Copper water)	Group 1	-0.15	0.13	0.249
	Group 2	0.83	0.13	<0.001
	Group 3	0.48	0.13	>0.001

Table 3: Tukey's post hoc multiple comparison test to assess level of significance between each group at the end of one hour (*Staphylococcus aureus*).

Candida albicans (First hour)	N	CFU/mL		p
		Mean	SD	
Gr 1 (Distilled water)	10	8.43×10^4	0.89	<0.001
Gr 2 (Vinegar)	10	0.62×10^4	0.05	
Gr 3 (Chlorhexidine)	10	1.39×10^4	0.13	
Gr 4 (Copper water)	10	0.08×10^4	0.03	

Table 4: The mean and standard deviation of colony forming units of *Candida albicans* between different experimental groups and control group at the end of one hour compared by one way ANOVA.

(I) Treatment		Mean Difference (I-J)	Std. Error	P
Group 1 (Distilled water)	Group 2	7.80	0.25	<0.001
	Group 3	7.03	0.21	<0.001
	Group 4	8.34	0.21	<0.001
Group 2 (Vinegar)	Group 1	-7.80	0.25	<0.001
	Group 3	-0.77	0.25	<0.001
	Group 4	0.54	0.25	<0.001

Group 3 (Chlorhexidine)	Group 1	-7.03	0.21	<0.001
	Group 2	0.77	0.25	<0.001
	Group 4	1.31	0.21	<0.001
Group 4 (Copper water)	Group 1	-8.34	0.21	<0.001
	Group 2	-0.54	0.25	<0.001
	Group 3	-1.31	0.21	<0.001

Table 5: Tukey's post hoc multiple comparison test to assess level of significance between each group at the end of one hour (*Candida albicans*).

At the end of six hours, all the experimental groups showed significant reduction in the number of S. aureus CFUs and maximum reduction was found in Group 4 with a mean value of $0.19x10^4$ CFU/mL. The least reduction was found in control group with mean value of $2.46x10^5$ CFU/mL (Table 6). Tukey's post hoc analysis revealed that between each group, the differences were not statistically significant ($p > 0.001$) (Table 7) (Figure 14).

Staphylococcus aureus After 6 hours	N	CFU/mL		p
		Mean	SD	
Gr 1 (Distilled water)	10	2.46×10^5	2.81	<0.015
Gr 2 (Vinegar)	10	0.20×10^4	0.04	
Gr 3 (Chlorhexidine)	10	0.37×10^4	0.04	
Gr 4 (Copper water)	10	0.19×10^4	0.06	

Table 6: The mean values of colony forming units obtained for *S. aureus* at the end of six hours when different groups were compared using one way ANOVA.

(I) Treatment		Mean Difference (I-J)	Std. Error	p.
Group 1 (Distilled water)	Group 2	2.26	0.72	.004
	Group 3	2.09	0.84	.019
	Group 4	2.26	0.89	.017
Group 2 (Vinegar)	Group 1	-2.26	0.72	.004
	Group 3	-0.17	0.84	.840
	Group 4	0.00	0.89	.994
Group 3 (Chlorhexidine)	Group 1	-2.09	0.84	.019
	Group 2	0.17	0.84	.840
	Group 4	0.17	0.98	.859
Group 4 (Copper water)	Group 1	2.26	0.89	.017
	Group 2	-0.00	0.89	.994
	Group 3	-0.17	0.98	.859

Table 7: Tukey's post hoc multiple comparison test to assess level of significance between each group of Staphylococcus aureus at the end of six hours.

At the end of six hours, statistically significant reduction in number of *C. albicans* CFUs were found in all the experimental groups and the maximum reduction was found in Group 3 (2% Chlorhexidine) with mean value of $0.08x10^4$ CFU/mL and least reduction was shown by the control group with mean value of $4.60x10^5$ CFU/mL (Table 8): Post hoc analysis revealed statistically significant difference between Groups 1 and 2, Groups 1 and 3, Groups 1 and 4 ($p < 0.001$) (Table 9) (Figure 16-18).

(I) Treatment		Mean Difference(I-J)	Std. Error	p
Group 1 (Distilled water)	Group 2	4.46	0.13	<0.001
	Group 3	4.51	0.14	<0.001
	Group 4	4.32	0.15	<0.001
Group 2 (Vinegar)	Group 1	-4.46	0.13	<0.001
	Group 3	0.05	0.15	.711
	Group 4	-0.13	0.15	.397
Group 3 (Chlorhexidine)	Group 1	-4.51	0.14	<0.001
	Group 2	-0.05	0.15	.711
	Group 4	-0.19	0.17	.273
Group 4 (Copper water)	Group 1	-4.32	0.15	<0.001
	Group 2	0.13	0.15	.397
	Group 3	0.19	0.17	.273

Table 9: Tukey's post hoc multiple comparison test to assess the level of significance between each group at the end of six hours (*Candida albicans*).

While comparing the results obtained at the time intervals of 1 hour and 6 hours after the immersion in different media, the statistical analysis (t-test) revealed that in Group I (Control) an overgrowth of CFUs of Staphylococcus aureus was found between the time intervals of 1 hour and 6hours and was statistically significant. But with Candida albicans there was reduction in CFUs between the time intervals. However, experimental groups showed reduction in both Candida albicans and staphylococcus aureus CFUs. From the data, significant difference was seen between control and the experimental groups. ($p < 0.001$) (Figure 17,18).

On assessing prolonged antibacterial action against staphylococcus aureus, only Group 1 showed an increase in number of colonies while all the experimental groups showed decrease in the number of CFUs. The differences were not statistically significant (p value > 0.001) (Table 10). The prolonged antifungal action against candida albicans was found in Group 3 (Chlorhexidine) and the mean values of CFUs decreased from $1.34x10^4$ at the end of first hour to $0.08x10^4$ at the end of sixth hour and the difference was significant ($p < 0.001$). A significant reduction in colony forming units were also observed in the control group and the mean values of colony forming units decreased from $8.43x10^5$ at the end of first hour to $4.60 x10^5$ at the end of sixth hour (Table 11). Other plates in group 2 and 4 showed reduction in both Candida albicans and staphylococcus aureus colonies. However, the differences were not statistically significant (p >0.001).

Discussion

Representation of the senior citizens in our population has increased in the last few decades and similarly the edentulism too has shown its significant presence. A considerable percentage of the edentulous individuals (67%) do suffer from denture stomatitis associated with Candida. Majority of the research work on stomatitis has focused on C. albicans because of the strong evidence received. Streptococcus and Staphylococcus species have also been related to denture stomatitis. Oral tissue facing the intaglio surface of the removable dentures are more prone to the deposition of the bacterial biofilm and denture stomatitis [26-28].

Denture hygiene has always been advised by dentists and the conventional practice is soaking in water or oxygen liberating effervescent cleansers, soaps and brushes. Brushes are capable of cleaning the dentures but care should be to avoid scratches which may increase biofilm deposition. Presently the denture hygiene requirement encompasses immersion of the dentures in solutions having antibiofilm/antimicrobial property. An ideal denture cleanser

Staphylococcus Aureus	N	CFU/mL				t	p
		One hour		Six hours			
		Mean	SD	Mean	SD		
Gr 1(Distilled water)	10	1.20×10^5	1.26	2.46×10^5	2.81	-1.398	.196
Gr 2 (Vinegar)	10	0.21×10^4	1.66	0.20×10^4	0.04	.143	.889
Gr 3 (Chlorhexidine)	10	0.70×10^4	3.33	0.37×10^4	0.04	2.296	.070
Gr 4 (Copper water)	10	1.04×10^4	0.02	0.22×10^4	0.10	14.735	.043

Table 10: Mean and standard deviation of number of CFU/ml of *S. aureus* at the end of one and six hours.

Candida albicans	N	CFU/mL				t	p
		1 hour		6^{th} hour			
		Mean	SD	Mean	SD		
Gr 1(Distilled water)	10	8.43×10^5	0.89	4.60×10^5	0.42	11.169	<0.001
Gr 2 (Vinegar)	10	0.62×10^4	0.05	0.13×10^4	0.13	7.408	.002
Gr 3 (Chlorhexidine)	10	1.34×10^4	0.06	0.08×10^4	0.06	26.704	<0.001
Gr 4 (Copper water)	10	0.07×10^4	0.03	0.27×10^4	0.29	-1.673	.170

Table 11: Mean and standard deviation of number of CFU/ml of *C. albicans* at the end of one and six hours.

should be able to remove biofilms, stains and deposits. The denture cleanser should be short acting i.e.. it should cleanse the denture within a period of 8 hours, duration which is commonly used to keep the denture immersed in water to prevent dimensional changes [29,30].

Majority of research workers have reached a consensus that the microbial composition of the biofilm obtained from teeth and dentures are similar. Studies in the past were based on cultures and presently scientists rely more on genome sequencing. In both systems of evaluations, a similarity is established on the microbial composition but some studies have contested the similarities [31]. The chemical present in mouth washes which are frequently used to control denture stomatitis can be effectively tried as a disinfectant denture cleanser.

The present study was designed including three products; a house hold material – white vinegar, an ayurvedic product – copper water made by storing water in copper vessels and an established pharmaceutical product – Chlorhexidine.

Chlorhexidine

Chlorhexidine is a widely accepted pharmaceutical product. It was developed in the UK by the Imperial chemical industry in the 1950s and later in the 1970s it was introduced in the US. Originally it was used as a surface disinfectant of clinics. It is effective against a wide variety of pathogens and in dentistry it was used as a mouth wash and denture disinfectant. It had a proven effect against C. albicans and denture stomatitis [32]. Chlorhexidine reduces bacterial adhesion when used as an overnight denture disinfection immersion solution [33,34]. In prosthodontic practice it is mainly employed in the disinfection of dentures and other acrylic based prostheses and impression trays. However, Chlorhexidine has limitations to be used in the disinfection of impressions because of the possibility of dimensional changes [35].

White vinegar

White vinegar is a household material used for making pickles, salads and cheese. Chemically it is 4-7% Acetic acid and the rest is water. The word vinegar has come from a French word 'vin aigre' which means sour wine and its use can be traced back to 5000 BC. It is produced from the fermentation of sugar or potatoes. White vinegar has antimicrobial properties expressed against food borne pathogenic bacteria. It has strong sanitizing effect at higher concentrations of 12% [36]. Keypetch R., *et al*. have tried vinegar as a denture cleansing agent and found its effectiveness against S. mutans and C albicans [37]. Silva Pinto., *et al.* have conducted an evaluation of candida levels in patients wearing complete dentures and suffering from stomatitis. The patients were asked to soak the denture in Vinegar overnight and found considerable reduction in the number of candida but complete elimination was not observed [38].

Copper

Copper is one of the abundant elements present on the earth crust and it has a history of usage that extends nearly five thousand years. In ancient times copper found a place in jewellery, coins and house hold utensils. In the 19th century, causal link between diseases and pathogens was found out and that triggered the exploration to find out antimicrobial agents. Many metals were tried as antibacterial agents and Copper had a prominent place. Research on copper and other metals were stalled in 1930 because of the introduction of antibiotics. Indiscriminate usage of antibiotics has resulted in the emergence of resistant variety of microorganisms like Methicillin resistant Staphylococcus Aureus (MRSA) and metals have again caught the attention of research workers for their antimicrobial potential. US environmental protection agency has recognised and assigned top priority to copper amongst metallic antimicrobial agents. Studies on the usage of copper as a denture disinfectant are not available. Probably the present study is the first of its kind [39-42].

Solid copper can impart antimicrobial effect through contact. Copper nano particles also have exhibited antimicrobial effect and it is attributed to DNA degradation that happens in a dose dependent manner in both gram positive and gram-negative organisms. When water is kept in a copper vessel for eight hours or more, copper releases ions into the water through oligodynamic effect and water gets antimicrobial effect. Ayurveda has recommended drinking water to be kept in copper vessel for its antimicrobial, anti-inflammatory and antioxidant properties. This water is designated as Copper water [43]. When copper ions accumulate inside the microbial cells, the oxidative behaviour of copper causes cell death by damaging the cell wall membrane. Release of copper ions cause oxidative stress by the production of reactive oxygen species (ROS). The ROS replaces or binds with the metalloproteins present in the cell wall of the microorganisms and eventually damages it [43-46].

To make the concept of denture hygiene more popular amongst the denture wearing elderly population especially those belonging to the economically weaker sections, it was decided to compare the antimicrobial potential of three immersion agents viz. White vinegar, Chlorhexidine and Copper water. Distilled water was included to serve as a control. Heat cure acrylic specimens were prepared (160). The specimens were inoculated with Staphylococcus aureus (80) and Candida albicans (80). After the formation of colonies (CFUs) the number was ascertained. The specimens were then immersed in the control and experimental media. After one hour, one set of specimens were evaluated for the number of CFUs and after six hours another set of specimens were evaluated for the CFUs. Results were analyzed using one way ANOVA and Tukey's post hoc multiple comparison (Figure 12).

At the end of one hour, the specimens treated with White vinegar, Chlorhexidine and copper water showed significant ($p < 0.001$) reduction in S. aureus colonies and the maximum reduction was observed with White vinegar. Similarly, C. albicans colonies also showed significant reduction of CFUs in the experimental groups. But the maximum reduction of CFUs were observed with Copper water. Control group had maximum number of CFUs with both S. aureus and C. albicans. Tukey's test showed distinct characteristics of the groups and the control group was far away from the experimental groups. The traditional immersion media like White vinegar and Copper water were efficient in controlling the growth of microorganisms similar to that observed with Chlorhexidine, a proprietary pharmaceutical product. These results also indicate the short acting capability of the immersion media. (Table 2-5).

At the end of six hours, the pattern of disinfection potential was similar to that observed at the end of 1 hour for S. aureus. Specimens treated with Copper water showed the least number of CFUs, followed by Vinegar and Chlorhexidine. Chlorhexidine exhibited the highest antimicrobial potency against Candida albicans, followed by Vinegar and Copper water. Tukey's post hoc analysis did not show significant differences between the groups. But in C. albicans, control group showed significant difference with all the experimental groups. Control group CFU value was $4.60x10^5$ CFU/mL but the values for Chlorhexidine, Vinegar and Copper water were $0.08x10^4$, $0.13x10^4$ and $0.27x10^4$ CFU/mL respectively. Immersion of dentures in antimicrobial media for 6 hours is a practical solution because disinfection is done during night when patients sleep. The control group CFU values were comparatively high ($4.60x10^5$ CFU/mL) indicating the fact that distilled water does not possess antimicrobial potency as expected and it was effective as a control (Table 6-9).

When the results obtained at the end of one hour immersion and six-hour immersion in denture cleansing agents used in the experimental groups and distilled water which was kept as a control, were compared the following findings were obtained. S. aureus CFUs showed an increase which can be attributed to the adaptable nature of Staphylococcus to osmotic balance and environmental stressors so that they could undergo cell division. CFUs of C. albicans controls showed a reduction at the same time intervals. It can be due to the osmotic stress caused by the hypotonic distilled water. All the experimental immersion media exhibited a lower CFU

value which was observed in the beginning. Statistically significant difference was evident between the time intervals only with C. albicans in the control and the experimental groups. With S. aureus, the results obtained between 1 hour and six hours was not significant. This comparison clearly indicates that White vinegar and Copper water have antimicrobial potential equivalent to Chlorhexidine and has the capability to maintain denture hygiene potential for six hours (Table 10,11).

Water has to be kept in copper vessel for at least eight hours for the effective release of copper ions. Denture wearers have to make a schedule of maintaining copper water in the house hold. It will be desirable to design a copper denture box which will serve both the purposes of storing denture in the night as well as maintaining ion levels in the copper water. It was observed that both white vinegar and copper water as effective antimicrobial denture cleansing media.

Conclusion

The following conclusions can be drawn from the observations of the present study:

- Copper water which is made by storing water in copper vessel for 6 - 8 hours can be used as an immersion disinfectant medium for acrylic prostheses.
- White vinegar is a potent disinfectant medium which can be used as an overnight storage medium for dentures.
- The present study endorses the already established fact that Chlorhexidine gluconate has antimicrobial effect which can be used as an overnight storage medium for dentures.
- All the three media mentioned above have antimicrobial effect against Candida albicans and Staphylococcus aureus which were used as reference organisms.
- For sustained antimicrobial effect acrylic prostheses should be stored in the above immersion media for six hours even though these media have quick acting potential gained within one hour.
- Following immersion, though disinfection happens, removal of deposits can be achieved only with supplementary brushing.

Limitations

Copper water and White vinegar can be studied for the antimicrobial potential against more strains of organisms. Side effects like discoloration and bleaching can be evaluated with long term storage.

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