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AN IN- VITRO EVALUATION OF ANTIMICROBIAL PROPERTIES OF TWO LUTING CEMENTS USED FOR FIXED PROSTHODONTICS CONTAINING ANTIBACTERIAL AGENTS

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ABSTRACT

Aims & Objectives: The aim of the present study is to evaluate and compare the effect of incorporation of Withania somnifera, Chlorhexidine digluconate and Syzygium aromaticum in Glass ionomer and Resin luting cements. **Materials & Methods:** In the present study the zones of inhibition of three different antimicrobial agents viz. Withania somnifera, Chlorhexidine digluconate and Syzygium aromaticum were evaluated by incorporating them into 200 disc shaped specimens of Glass Ionomer Cement [Gold Label, GC corporation, Tokyo, Japan] and 200 disc shaped specimens of Panavia F 2.0 dual cure resin cement [Kuraray]. **Results:** The present study demonstrated that Withania somnifera, Chlorhexidine digluconate and Syzygium aromaticum added to glass ionomer cement and resin luting cement resulted in compounds with increased antibacterial effect as compared to the cements alone. Although the glass ionomer cement and resin luting cement inhibited bacterial growth slightly, the addition of Withania somnifera, Chlorhexidine digluconate and Syzygium aromaticum increased the antibacterial activity of the cements by four to ten fold. **Conclusion:** From the results of our study it can be concluded that the plant extracts as well as Chlorhexidine have antimicrobial activity when incorporated in luting cements. Although they have the potential to be used for luting crowns and bridges. However, future research efforts are needed for the evaluation of bond strength of the plant products for their regular use in the oral cavity.

KEYWORDS: Withania somnifera, Chlorhexidine digluconate and Syzygium aromaticum.

INRTODUCTION

Dental luting agents provide link between fixed prosthesis and supporting prepared tooth structure.[1] These luting agents may develop gap formation along the line of restoration and can cause microleakage due to varied stress factors. These gaps can inhabit microorganisms to colonize, thereby promoting secondary caries formation. Secondary caries being one of the major factors in the failure of fixed prosthesis. Pathogenesis of dental caries suggests that Streptococcus mutans (S. mutans) metabolize sucrose more efficiently than other common oral bacteria, thus it becomes an important resident in caries- associated biofilms. Hence, study on inhibitory functions of the material against bacterial growth is of paramount importance to be chosen as the best material in preventing secondary caries formation.[2] In attempting to study these effects, several investigators have evaluated the antimicrobial action of dental cements and have generally concluded that all the tested materials had some bacteriostatic effect. Various studies have incorporated different antimicrobial agents in luting cements showing potential antimicrobial activity against Streptococcus mutans strains for added benefits.

A widely adopted approach to prevent dental caries is the application of chemoprophylactic agents.[3] These agents like Chlorhexidine have been incorporated in luting cements to enhance their antibacterial action.

Natural products have also been used as a major source of innovative and effective therapeutic agents in the dental field for preventing oral diseases, particularly dental biofilm related diseases, such as caries. Recently, several studies have shown that some natural products can interfere with the survival and virulence properties of mutans streptococci.[4]

Withania somnifera, also known as ashwagandha or winter cherry, is a plant of the Solanaceae family. Previous reports on the activities of methanolic extracts of the W. somnifera plant against different pathogenic bacteria have found significant antibacterial properties.[5]

Another natural product, clove (Syzygium aromaticum) has proven to be effective against many different types of bacteria.

However the literature about antimicrobial activity of Chlorhexidine digluconate, Withania somnifera and Syzygium aromaticum when mixed with dental luting cements is scanty. Therefore a study was conducted to evaluate and compare the antimicrobial properties of Withania somnifera, Chlorhexidine digluconate and Syzygium aromaticum incorporated in Glass Ionomer cement and Dual cure resin luting cement against Streptococcus mutans.

MATERIAL AND METHODS

In the present study the zone of inhibition of three different antimicrobial agents viz. Withania somnifera, Chlorhexidine digluconate and Syzygium aromaticum were evaluated by incorporating them into 200 disc shaped specimens of Glass Ionomer Cement [Gold Label, GC corporation, Tokyo, Japan] and 200 disc shaped specimens of Panavia F 2.0 dual cure resin cement [Kuraray].

For the preparation of the discs a metallic mould was fabricated consisting of a base, a middle plate containing hollowed out spaces of 5mm inner diameter and 2mm thickness, and a lid (FIGURE 1).

Preparation of Withania somnifera extract

Withania somnifera roots were washed thoroughly in running tap water followed by distilled water to remove dust and contaminants. These were then shade dried and pulverized into fine powder using a sterilized grinder. 100gm of this powder was measured on a weighing scale and mixed with 300ml of methanol by stirring followed by sonication or ultrasound assisted extraction (UAE) for 10 minutes. This process was repeated three times so that the mechanic effect of acoustic cavitation from the ultrasound increases the surface contact between methanol and the powder particles facilitating release of compounds from it. The solvent was evaporated under reduced pressure in a rotary evaporator to obtain a methanolic extract of Withania somnifera.

Preparation of Syzygium aromaticum extract

Syzygium aromaticum buds were washed thoroughly in running tap water followed by distilled water to remove dust and contaminants. These were then shade dried and pulverized into fine powder. 100gm of this powder was measured on a weighing scale and mixed with 300ml of methanol by stirring followed by sonication or ultrasound assisted extraction (UAE) for 10 minutes. This process was repeated three times. The solvent was evaporated under reduced pressure in a rotary evaporator to obtain a methanolic extract of Syzygium aromaticum .

Preparation of agar plate cultures

Standard strains of Streptococcus mutans (MTCC 497) in the form of lyophylized culture were obtained and used to test antimicrobial efficacy. These microorganisms were grown in 5ml of Brain Heart Infusion broth for 24 hours at 37°C in an incubator to form an inoculum. The bacterial colonies were taken from the broth cultures and adjusted to 0.5 Mc Farland standard. Blood agar was spread evenly to a thickness of 5mm in petridishes and after solidification the agar plates were dried and the bacterial culture was spread onto them and incubated in a candle jar. Seven wells of diameter 5mm and 2mm depth were made in each agar plate. These agar plates were then incorporated with luting material discs of same dimensions.

Preparation of discs

400 Disc shaped specimens were made of Glass Ionomer cement and dual cure resin luting cement as follows.

Group 1: The zone of inhibition was checked by preparing 200 Glass Ionomer Cement discs.

Subgroup 1a - 50 disc shaped specimens (5 x 2 mm) of Glass Ionomer cement were prepared under aseptic conditions in a laminar airflow chamber. The powder and liquid of Glass Ionomer cement was mixed according to the manufacturers instructions. The middle plate of the metallic mould was seated on the base and a thin coat of vaseline was applied with a cue tip applicator as a separating medium on the inner diameter of the holes (Figure 1). The mixed cement was placed in the holes after which the lid was closed. The cement was allowed to set and then the discs were retrieved.

Subgroup 1b - 50 disc shaped specimens (5 x 2 mm) were prepared by incorporating 0.28% w/w of Withania somnifera extract in Glass ionomer cement.

Subgroup 1c - 50 disc shaped specimens (5 x 2 mm) were prepared by incorporating 10% w/w of Chlorhexidine digluconate in Glass ionomer cement.

Subgroup 1d - 50 disc shaped specimens (5 x 2 mm) were prepared by incorporating 0.17% w/w of Syzygium aromaticum extract in Glass ionomer cement.

Group 2: The zone of inhibition was checked by preparing 200 dual cure resin luting cement discs.

Subgroup 2a - 50 disc shaped specimens (5 x 2 mm) of dual cure resin luting cement were prepared under aseptic conditions in a laminar airflow chamber. Tube A and tube B was mixed according to the manufacturers instructions. The middle plate of the metallic mould was seated on the base and a thin coat of vaseline was applied with a cue tip applicator as a separating medium on the inner diameter of the holes. The mixed cement was placed in the holes after which the lid was closed. The cement was allowed to set chemically and then light cured. The set discs were then retrieved.

Subgroup 2b - 50 disc shaped specimens (5 x 2 mm) were prepared by incorporating 0.28% w/w of Withania somnifera extract in dual cure resin luting cement.

Subgroup 2c - 50 disc shaped specimens (5 x 2 mm) were prepared by incorporating 10% w/w of Chlorhexidine digluconate in dual cure resin luting cement.

Subgroup 2d - 50 disc shaped specimens (5 x 2 mm) were prepared by incorporating 0.17% w/w of Syzygium aromaticum extract in dual cure resin luting cement.

Incubation period

All the discs were placed in the wells punched in the blood agar. The petridishes were inverted, placed in a candle jar and incubated anaerobically in an incubator for 24 hours at 37°C.

Measurement of antibacterial activity

After the incubation period, the petri dishes were removed from the incubator and the diameter of zone of inhibition was measured for each disc at two different points perpendicular to each other with the help of vernier calipers. The mean of the two readings was calculated.

RESULTS AND DISCUSSION

Control of dental plaque is the most important factor in caries prevention.[1] Restoration margins can provide a potential pathway to leakage of cariogenic microorganisms present in the normal human flora.[6] Cariogenic microbiota is composed mainly of streptococci, lactobacilli and actinomyces among which mutans group streptococci, like S. mutans and S. sobrinus, are considered as primary etiologic agents in secondary caries associated with fixed prosthesis.[7] This is the reason why S. mutans was chosen for inoculum in this study.

Among all dental materials, glass ionomer cements are reported to be the most cariostatic. Growth of Streptococcus mutans has been reported to be inhibited in vivo around Glass ionomers, which has generally been attributed to fluoride released by the material. Various bacteriostatic and bactericidal agents have the potential to be used in combination with glass ionomer cements to obtain a synergistic antibacterial restorative material.[8]

Resin cements are also now routinely used for the cementation of indirect tooth-colored restorations and have the ability to bond to both tooth structure and restorations. In order to prevent secondary caries, it is important that the resin cements used in dentistry should have an antibacterial effect against cariogenic bacteria.[9]

The present study was conducted by adding 0.28% w/w Withania somnifera, 10% w/w Chlorhexidine digluconate and 0.17% w/w Syzygium aromaticum in Glass Ionomer luting cement and dual cure resin luting cement.

Withania somnifera, popularly known as Ashwagandha is a well known medicinal plant. It has bactericidal activity against S. mutans and S. sobrinus in the planktonic state.[4] However, little information is available on the activity of W. somnifera against the bacteria in dental luting cements.

Chlorhexidine is regarded as the contemporary gold standard frequently employed in oral care. It has been used in the past in various concentrations to improve antimicrobial activity of Glass ionomer and Resin cement. Chlorhexidine digluconate was used in the study because it demonstrated significantly more inhibition than chlorhexidine dihydrochloride for Streptococcus mutans.[10] Major characteristics of chlorhexidine that contribute to its success as an antiplaque agent include its substantivity and broad spectrum of antibacterial activity.[11] It is associated with the promotion of hybrid layer and improvement of physical properties of resin cement. It also diminishes the loss of bonding effectiveness over time associated with etch and rinse and self etch cements and also reduces microleakage at gingival margin after storage.[12,13] The antibacterial effect of Chlorhexidine is concentration dependent. Adding chlorhexidine digluconate to luting cement has been shown, however, to change the mechanical properties with the 10% addition producing mechanical properties nearest to those of the unmodified products. This cement formulation could be useful in reducing decalcification, periodontal disease around the crown margins and in overcoming problems of cooperation with adherence to use of an antimicrobial mouth rinse regime after fixed prosthodontic treatment.[14]

Cloves (Syzygium aromaticum) are dried aromatic unopened floral buds of an evergreen tree.[15] They have potent antibacterial properties against Streptococcus mutans and therefore their use in combination with luting cements has been evaluated here.

The present study demonstrated that Withania somnifera, Chlorhexidine digluconate and Syzygium aromaticum added to glass ionomer cement and resin luting cement resulted in compounds with increased antibacterial effect as compared to the cements alone. Although the glass ionomer cement and resin luting cement inhibited bacterial growth slightly, the addition of Withania somnifera, Chlorhexidine digluconate and Syzygium aromaticum increased the antibacterial activity of the cements by four to ten fold.

Upon comparing the results between the subgroups of Group 1 i.e. Glass ionomer luting cement, it was found that the values of the mean zone of inhibition of Glass ionomer cement discs with Chlorhexidine digluconate (Subgroup 1c) were the highest (10.72 ± 1.08 mm) followed by Glass ionomer cement discs with Syzygium aromaticum (Subgroup 1d) (7.19 ± 0.56 mm), Glass ionomer cement discs with Withania somnifera (Subgroup 1b) (4.56 ± 0.66 mm) and Glass ionomer cement discs without antimicrobial agent (Subgroup 1a) (1.48 ± 0.61 mm). (TABLE 1, GRAPH 1, FIGURE 2).

The results of Chlorhexidine digluconate was in accordance with a previous study by **de Morais Sampaio GA et al** who stated that the antimicrobial effects shown by 10% Chlorhexidine digluconate addition to Glass ionomer cements were sufficient to provide protection against Streptococcus mutans.[16]

Similar results were obtained in the study by **Jedrychowski JR et al** who determined the antibacterial characteristics and effects on mechanical properties when chlorhexidine gluconate or chlorhexidine dihydrochloride were added to a composite resin and a glass ionomer restorative material in different concentrations. It was concluded that the addition of chlorhexidine gluconate or chlorhexidine dihydrochloride increased the antibacterial activity of the composite resin and the glass ionomer restorative material.[10]

The slightly higher antibacterial activity of Syzygium aromaticum in comparison to Withania somnifera can be attributed to the fact that it has a lower minimum bactericidal concentration which makes it a better antimicrobial agent against Streptococcus mutans.

The surface adherence ability of Streptococcus mutans may be affected by the presence of clove extracts in the luting cements. According to **Abd Rahim ZH et al** this may be due to the alteration of the initial attachment of Streptococcus mutans.[17] They compared the efficiency of crude aqueous (CA) and solvent extracts (CM) of clove on the caries-producing properties of Streptococcus mutans. The two extracts were found to reduce the synthesis of water-insoluble glucan by almost 50% at a concentration as low as 0.5 mg/ml and the solvent extract exhibited a significantly higher inhibitory effect than the aqueous extract ($P < 0.05$). Their findings indicated that both the aqueous and solvent extracts exert inhibitory effects on the cariogenic properties of S. mutans.

According to **Kharel P et al** and **Sundaram S et al** the antimicrobial properties of Withania somnifera may be attributed to the withanolide compounds present in it.[18,19]

However, the data on the activity of W. somnifera and Syzygium aromaticum against the bacteria in Glass Ionomer luting cement is scanty.

In Group 2 i.e. resin luting cement group, the mean zone of inhibition of discs with Chlorhexidine digluconate was the highest (9.93 ± 0.91 mm) followed by discs with Syzygium aromaticum (6.15 ± 1.13 mm) which were almost similar to discs with Withania somnifera (5.73 ± 0.42 mm) with p value = 0.070. These were followed by Resin luting cement discs without an antimicrobial agent (0.87 ± 0.78 mm). (TABLE 2, GRAPH 2, FIGURE 3).

The antibacterial effect of Chlorhexidine added to Resin cement was established in a previous study by **Singh Y et al** who evaluated the effect of 2% Chlorhexidine on antibacterial activity on Resin cement. They stated in their study that chlorhexidine was associated with promotion of hybrid layer and improvement of physical properties of Resin cement. It also diminished the loss of bonding effectiveness over time associated with etch and rinse and self etch cements and also reduced microleakage at gingival margin after storage.[13]

However, the data on the activity of Withania somnifera and Syzygium aromaticum against the bacteria in Resin luting cement is scanty.

Glass ionomer cement without any addition of antimicrobial agent showed slightly more antibacterial action than dual cure resin cement without any additional antimicrobial agent (0.61±0.24 mm). The antibacterial action of glass ionomer cement with Withania somnifera was slightly less than that of dual cure resin cement with Withania somnifera (1.17±0.17 mm). Glass ionomer cement discs with Chlorhexidine digluconate showed more bacterial inhibition than resin luting cement with Chlorhexidine digluconate (0.79±0.17 mm). Syzygium aromaticum incorporated in glass ionomer cement also had slightly higher antibacterial action than Syzygium aromaticum incorporated in resin luting cement (1.04±0.57 mm). The results were statistically significant showing that Glass ionomer cement had higher antimicrobial activity than dual cure resin luting cement with or without the addition of antimicrobial agents. (TABLE 3, GRAPH 3).

The higher antimicrobial activity of Glass ionomer cement may be attributed to the fluoride release capability and the acidity of the Glass ionomer cement.[3]

The highest amount of antimicrobial activity with mean inhibitory zone was found in Glass ionomer cement with Chlorhexidine digluconate followed by Resin cement with Chlorhexidine digluconate, Glass ionomer cement with Syzygium aromaticum, Resin cement with Syzygium aromaticum, Resin cement with Withania somnifera, Glass ionomer cement with Withania somnifera, Glass ionomer cement and Resin cement in decreasing order.

The incorporation of chlorhexidine in Glass ionomer cement acts in synergy with its fluoride- releasing and substantivity properties.[3]

Table 1: Mean comparisons among Group 1a, 1b, 1c and 1d zones of inhibition.

Groups	Min	Max	Mean	SD	P value	Result
Group 1a	0.45	2.70	1.48	0.66	0.000	Significant
Group 1b	3.60	5.80	4.56	0.61		
Group 1c	9.50	15.15	10.72	1.08		
Group 1d	6.50	8.20	7.19	0.56		

Statistical Analysis: ANOVA one way test. Statistically significant if $P<0.05$; $p<0.001$; highly significant
SD : Standard deviation.

Table 2: Mean comparisons among Group 2a, 2b, 2c and 2d Zones of inhibition.

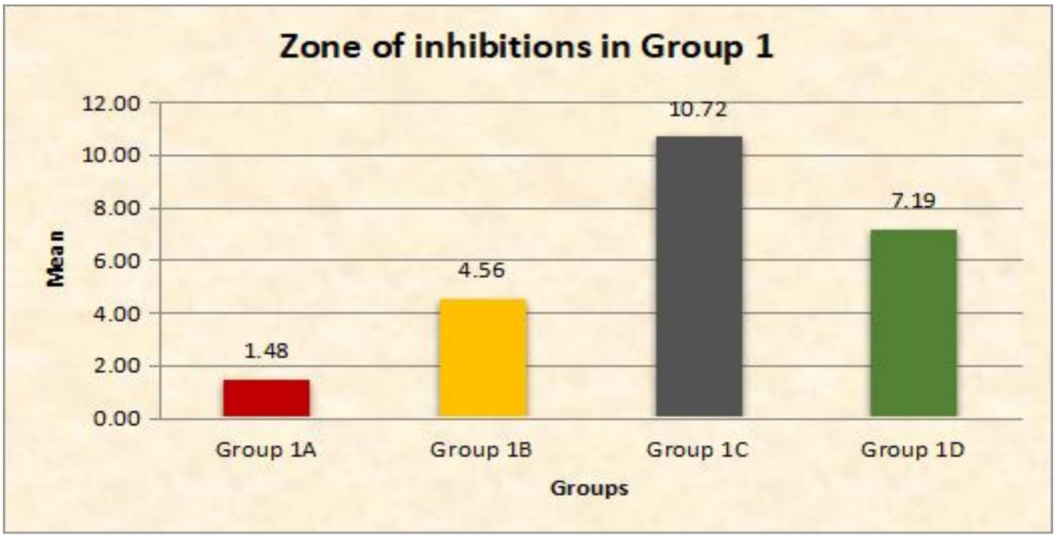
Groups	Min	Max	Mean	SD	P value	Result
Group 2a	0.10	1.80	0.87	0.42	0.000	Significant
Group 2b	4.35	7.30	5.73	0.78		
Group 2c	7.70	11.40	9.93	0.91		
Group 2d	4.00	8.15	6.15	1.13		

Statistical Analysis: ANOVA one way test. Statistically significant if $P<0.05$; $p<0.001$; highly significant, SD: Standard deviation.

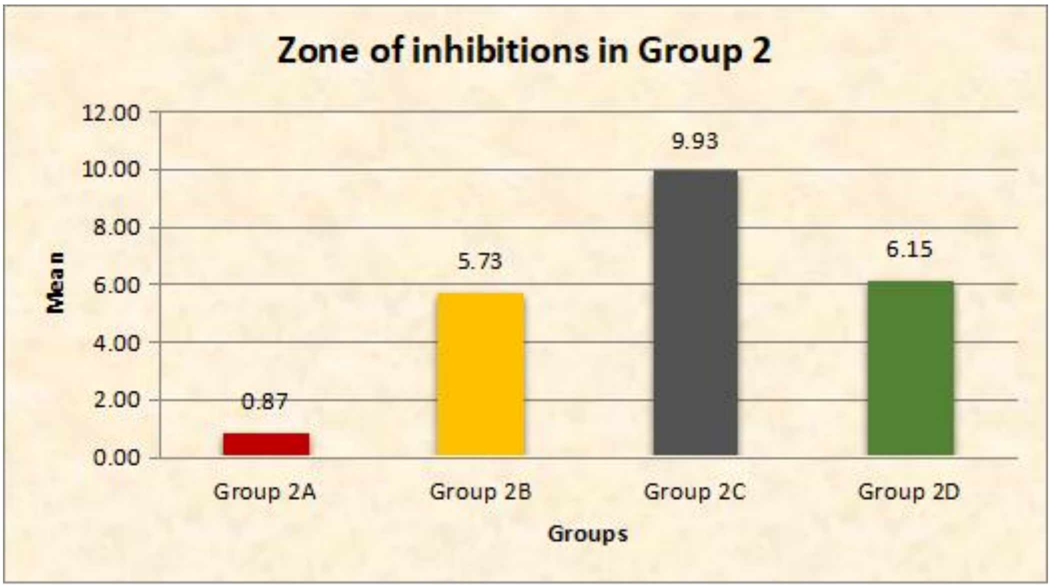
Table 3: Mean comparisons between Group 1 Vs Group 2 Zones of inhibition.

Groups	Mean	SD	Difference Mean±SD	P value	Result
Group 1a	1.48	0.66	0.61±0.24	0.000	Significant
Group 2a	0.87	0.42			
Group 1b	4.56	0.61	1.17±0.17	0.000	Significant
Group 2b	5.73	0.78			
Group 1c	10.72	1.08	0.79±0.17	0.000	Significant
Group 2c	9.93	0.91			
Group 1d	7.19	0.56	1.04±0.57	0.000	Significant
Group 2d	6.15	1.13			

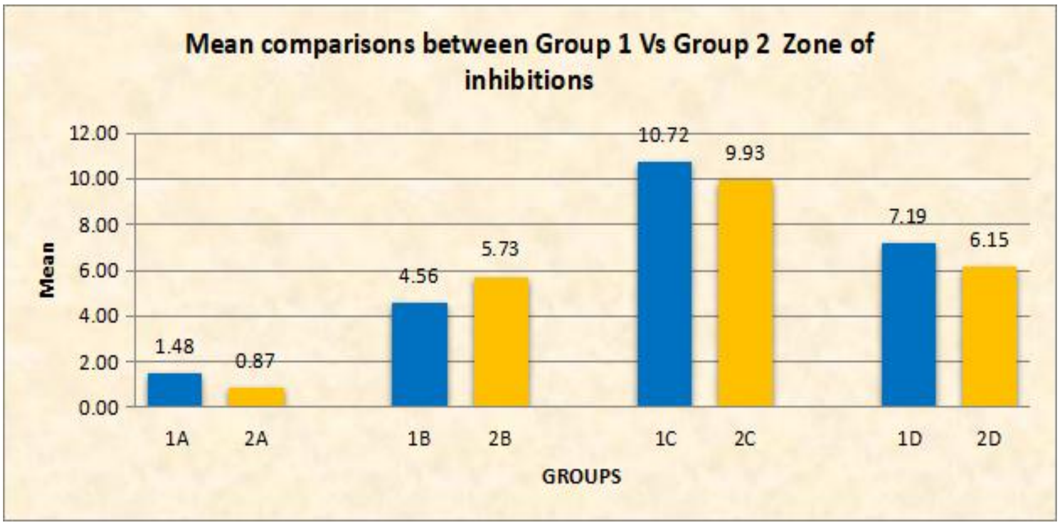
Statistical Analysis: Independent sample t test. Statistically significant if $P<0.05$; $p<0.001$; highly significant.



Graph 1: Mean comparisons among Group 1a, 1b, 1c and 1d zones of inhibition.



Graph 2: Mean comparisons among Group 2a, 2b, 2c and 2d Zones of inhibition.



Graph 3: Mean comparisons between Group 1 Vs Group 2 Zones of inhibition.

LEGENDS

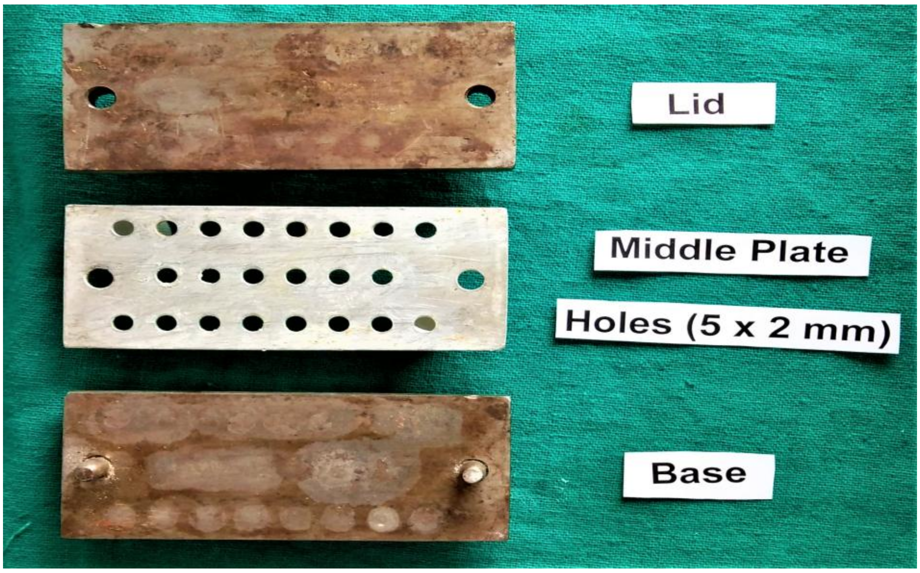


Figure 1: Metallic mould.

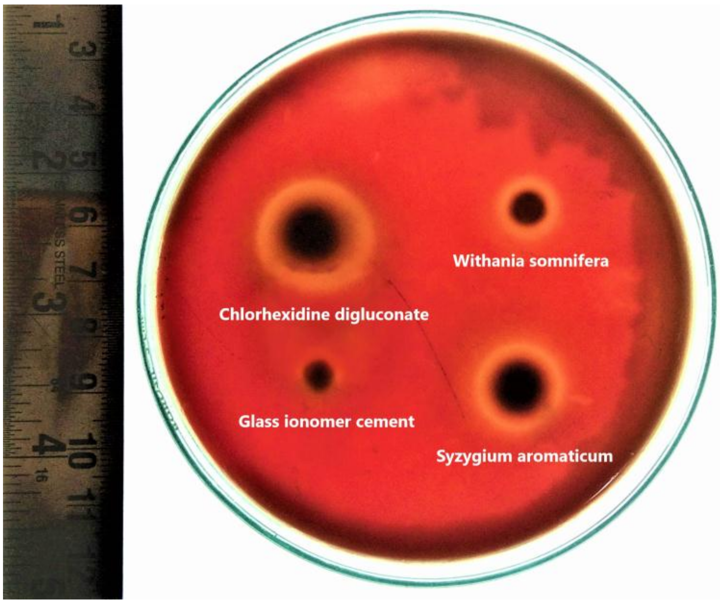


Figure 2: Zones of inhibition of Glass ionomer cement, Glass ionomer cement with Withania somnifera, Glass ionomer cement with Chlorhexidine digluconate and Glass ionomer cement with Syzygium aromaticum.

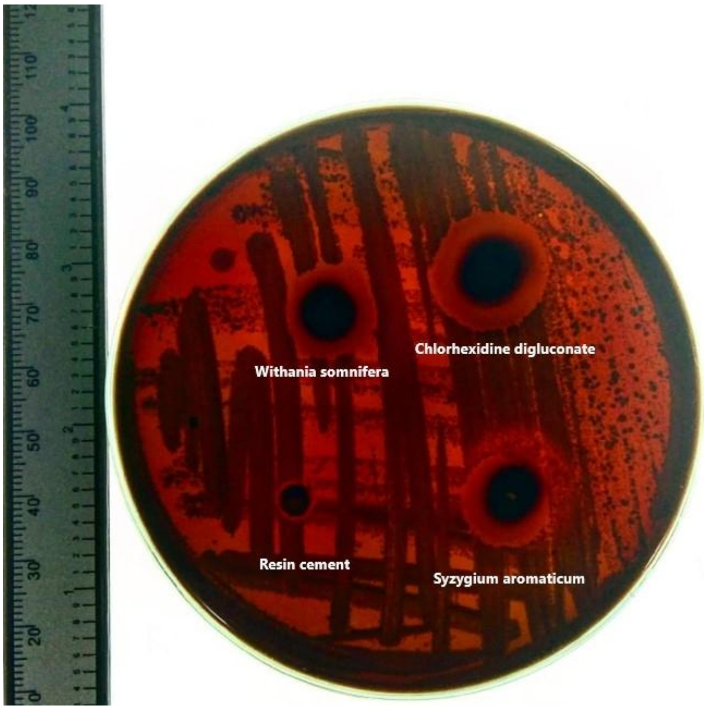


Figure 3: Zones of inhibition of Dual cure resin cement, Dual cure resin cement with Withania somnifera, Dual cure resin cement with Chlorhexidine digluconate and Dual cure resin cement with Syzygium aromaticum.

CONCLUSION

We found out that all groups with antimicrobial agents showed strong activity against Streptococcus mutans as compared to the control group.

The highest amount of antimicrobial activity with mean inhibitory zone was found in Glass ionomer cement with Chlorhexidine digluconate followed by Resin cement with Chlorhexidine digluconate, Glass ionomer cement with Syzygium aromaticum, Resin cement with Syzygium aromaticum, Resin cement with Withania somnifera, Glass ionomer cement with Withania somnifera, Glass ionomer cement and resin cement in decreasing order.

Thus from the results of our study it can be concluded that the plant extracts as well as Chlorhexidine have antimicrobial activity when incorporated in luting cements. Although they have the potential to be used for luting crowns and bridges. However, future research efforts are needed for the evaluation of bond strength of the plant products for their regular use in the oral cavity.

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