Incorporation Of Ajwain Extract in Provisional Luting Cement for Long Term Inhibition of Bacterial Infiltration

Madhura Deshmukh1, Subhabrata Maiti2*, Rajeshkumar S3, Dhanraj Ganapathy4
1Department of Prosthodontics and Implantology, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai-600077, Tamilnadu, India, Email: madhuradesh1294@gmail.com
2Assistant Professor, Department of Prosthodontics and Implantology, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai-600077, Tamilnadu, India, Email: drsubhoprostho@gmail.com
3Associate Professor, Department of Pharmacology, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai-600077, Tamilnadu, India
4Professor and head of department, Department of Prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, Tamil Nadu, India

Abstract

Aim: The focus of this study was to evaluate the antimicrobial properties of Ajwain seeds when incorporated in provisional luting cement.

Materials and Methods: A total of 12 samples of provisional cement [TEMP-BOND™] were prepared. Extraction from 5gms weighed Ajwain seeds with 100ml distilled water was done until reduced to 10ml. Minimum Inhibitory Concentration (MIC) was determined using agar diffusion broth method against Streptococcus Mutans and Candida Albicans. Independent t test was conducted to assess the level of significance of difference between mean values.

Results: For both Streptococcus Mutans and Candida Albicans, the difference between two methods (Incorporated or Dipped/Coated) showed statistically significant difference (p<0.05) in antimicrobial activity and both the methods are clinically acceptable as it had adequate zone of inhibition.

Conclusion: The zones of inhibition in the agar diffusion test showed the antibacterial efficiency of provisional luting cement when incorporated or dipped with ajwain extract is more than the control group and Ajwain can be considered as a promising natural source of antibacterial agent for management of dental secondary caries.

Keywords: Ajwain extract, provisional luting cement, anti-microbial properties, long term temporisation, oral health

DOI: 10.47750/pnr.2022.13.S07.068

INTRODUCTION

Oral health and hygiene marks the window to each individual’s overall health. Oral diseases triggered by pathogenic bacteria persist as a worldwide problem with higher impact on human health.(1–3)Well-maintained oral health contributes positively to one’s physical and social well-being. In developing countries, cavities remain the foremost important dental ill health and various strategies are being developed for its effective prevention and management. Dental caries being transmissible may be a multifactorial disease caused thanks to the occurrence and interaction between dental biofilm and oral microflora. In major prosthetic cases like full mouth rehabilitation, patients are given long-term temporization. Temporary crowns provide favorable conditions for the colonization of microorganisms. Primary colonizers of the microflora like Streptococcus mutans are liable for plaque formation. Thus, luting cements with an antimicrobial additive will have profound benefits in providing a seal and preventing caries formation. Various natural agents have proven their efficacy over chemotherapeutic agents in terms of antibacterial activity, mainly thanks to polyphenol as its major constituent. Trachyspermum Ammi or Carum copticum, commonly referred to as Ajowan or Ajwain may be a plant used as a spice. ‘Thymol’(35-60%) , the main phenolic constituent present in Ajwain seeds is responsible for its antibacterial and germicidal effect.(1,4) It's also used for cleaning wounds and treating skin infections.

As of now, wherein the shift of health care approaches from synthetic products to natural product alternatives is observed. Traditional Indian medicines are suggested to be potential options in combating cavity causing microorganisms. One out of the
Ajwain is traditionally well-known for its diverse therapeutic uses especially the oil exhibits antimicrobial, anti-inflammatory, antispasmodic, and antiplatelet activity.[(5)] The various dental luting cements which are used link the fixed dental restoration and tooth structure. Conventionally, the supply of all luting cements used for interim restoration should have the perfect features like the provision of a gap-free interface, and also the prevention of micro-leakage and secondary caries. This helps to decrease the failure rates of prostheses.

Two significant microflora-causing cavities are Streptococcus mutans (SM) and Lactobacillus Casei (LB) and that they are considered the primary colonizers. A decrease within the levels of those microorganisms may be a viable preventive strategy to combat cavity progression. Thus, luting cements with an antimicrobial additive will have profound benefits in providing a seal and preventing caries formation. Recently, a growing interest has been observed toward the use of traditional herbal medicine alongside synthetic modern drugs (6) as they have similar efficacy as chemotherapeutic agents with none side effects.

Long-term temporization is required for fully mouth rehabilitation (FMR) cases and periodontally compromised cases. Thus, antibacterial modified interim cements act as powerful antimicrobial agents. They control the marginal space biofilm which prevents the initial colonization of cariogenic bacteria. In previous studies, Ajwain oil has comparable antibacterial activity against oral bacteria than chlorhexidine and also significantly higher bacteriostatic and bactericidal efficacy at an equivalent concentration of chlorhexidine. (7) Also, the fungitoxicity of the oil was found to be thermo-stable up to 150°C and thymol was identified as the fungitoxic chemical in essential oil. (6,8)

Keeping all the factors into consideration, we conducted an in vitro study to gauge the antimicrobial efficacy of Ajwain extract against Streptococcus mutans and Candida albicans. Our team has extensive knowledge and research experience that has translated into high quality publications (9–23).

This study aimed to gauge the antibacterial activity, the minimum inhibitory concentration of provisional luting cement (here, TEMP-BOND™) after the addition of Ajwain extract.

**Material And Methods**

**Sample And Preparation Of Ajwain Extract**

A fresh package of Ajwain seeds was bought from the local supermarket and was used for the subsequent study. The extraction from Ajwain seeds was administered using Soxhlet apparatus. 5gms of Ajwain seeds were weighed and extracted with 100ml of water. Constant heat was provided. Solution was stirred continuously and evaporated until its colour changed for about 15 mins. This solution was re-heated to scale back the extract to 10ml. Later, the extract was transferred into a clean, sterile and pre-weighed beaker. The semi-solid extract was stored at 4 degrees C in sterile and labelled plastic tubes until further use.

**Minimum Inhibitory Concentration(MIC)**

Minimum Inhibitory Concentration(MIC) decided using agar diffusion broth method. Pure cultures of Streptococcus Mutans and Candida albicans were grown in agar broth separately. The microtiter plates were incubated at ideal temperatures for 18-24hrs and thereafter observed for growth or turbidity. The agar plates were examined for growth using the unaided eye.

**Sample Preparation**

6 samples per group (Group1: Incorporated, Group 2: Dipped, Group 3: Control) of provisional cement [TEMP-BOND™] were prepared. Powder liquid ratio of luting cement used was 30g(base paste) and 13g(catalyst paste) which was added to 1 ml of extract of Ajwain. A mould before mixing was prepared with dimensions of sample size 5mm radius circle and 2mm thickness. Individual TEMPBOND™ mixture samples (which consists of accelerator and base paste) were either dipped or incorporated with ajwain extract or used as an impact group. For incorporation, Ajwain extract was directly mixed along side luting cement and allowed to line. For dipping or coating, the luting cement was mixed and allowed for initial set, followed by partial then dipped into Ajwain extract and waited until complete set.

Test microorganisms were subcultured on selective media and incubated aerobically at ideal temperature for twenty-four hrs. a complete of three wells were made onto each plate. Plate 1 consisted of cultured Streptococcus Mutans microorganisms. Plate 2 consisted of cultured Candida albicans microorganisms. Each sample for every group was placed within the respective wells and were labelled. Plates were incubated at 37°C for twenty-four hrs in an aerobic environment. After overnight incubation,
plates were observed for the zone of inhibition and diameter of the inhibition zone. These were measured using measuring tape at four different points and mean was calculated.

Statistical analysis for values of zone of inhibition was administered using IBM SPSS software (version 23). Independent t test was conducted to assess the extent of significance of difference between mean values.

Results

Both Streptococcus Mutans and Candida Albicans demonstrated an adequate anti-microbial activity when the temporary cement was mixed with ajwain extract using both methods of incorporation and dipped/coated, whereas, the control group did not show any zone of inhibition. In Gram positive bacterial group Streptococcus Mutans had minimum zone of inhibition in Ajwain extract incorporated into TEMPBOND (15.0±2.0) whereas Ajwain dipped/coated onto TempBond showed maximum zone of inhibition (27.66±2.51). Next most common fungal organism causing oral infections under this group was Candida Albicans which had minimum zone of inhibition in the Ajwain extract incorporated into temporary cement(15.66±2.51) and Ajwain dipped/coated with temporary cement showed maximum zone of inhibition (35.33±2.51) respectively. For both the microbes, the difference between two methods was showing significant difference (p<0.05) in antimicrobial activity but both the methods were clinically acceptable as it was showing a good amount of zone of inhibition.

Discussion

It is impossible to eliminate all bacteria development completely from the oral cavity in any preventive approach. But, there is a possibility of development of secondary caries with the use of temporary prosthesis for longer duration inside the mouth. Therefore, it is important to add an antibacterial agent to the provisional luting cement used. Ajwain extract was chosen as the antibacterial agent for this new provisional cement. The provisional cement TEMP-BOND™ is primarily used as a luting cement for temporary prosthesis. It can stay in the oral cavity for a longer duration of time as in cases of full-mouth rehabilitation and periodontally compromised cases. Extraction of antibacterial agent was done from ajwain seeds in the laboratory using the Soxhlet method which is considered as the standard one. Common factors affecting the extraction process include seed properties, solvent, temperature and time.

Previous studies by Mahesh et al (7), stated that ajwain can serve as a potential, natural, non-toxic and economical therapeutic anti-plaque agent. A study by Batool et al(24)also [(25,26)], shows that ajwain oil has antibacterial effects against human pathogens.In a study by Namrata O et al (27), it was found that Ajwain oil cures tooth pain and when used along with equal parts of clove oil and water acts as home remedy to floss mouth, preventing tooth decay. In a previous study by Tuzuner et al (28) Streptococcus mutans being a primary colonizer in the oral cavity is considered as bacteria with high cariogenic potential because of its acidogenicity and aciduricity(29). Khan et al. reported that antibacterial action of T. ammi extract on S. mutans is due to (4As, 5R,8aS)5,8a-di-1-propyl-tetrahydronaphthalen-1-(2H)-1 a novel compound. This compound showed its activity against S. mutans by significantly reducing its adherence as well as biofilm formation, insoluble glucan synthesis GF and hydrophobicity. (29) Ajwain seed oil has also been found to kill Candida albicans in vitro, according to some researchers, possible by membrane inhibition. (5) (30) However, as far as we know, the anti-Candida effect of aqueous extract of seeds of Ajwain, as also its anti-biofilm activity on C. albicans, have not been yet assessed or published in the literature. Candida albicans readily forms biofilms on dentures surfaces, which provide a surface for yeast growth and often lead to refractoriness to available antifungal therapy. In this study, the overall results show highest MIC values for dipped (Group 2) against Candida Albicans. Given the emerging high degree of resistance of Candida albicans to antifungal drugs, ajwain extract definitively is a promising alternative since it could inhibit both growth and biofilm formation. It was also safe, and this toxicity assay can also be done in cell lines for better results. (31)

Limitation of the present study is that evaluation of physical and mechanical properties of provisional luting cement has to be done. Also, for other major caries causing microorganisms', antimicrobial activity to be evaluated.

Conclusion

Within the limitations of the study, it was concluded that the zones of inhibition in the agar diffusion test showed the antibacterial efficiency of provisional luting cement used when incorporated or dipped with ajwain extract is more than the control group. Thus, Ajwain can be considered as a promising natural source of antibacterial agent for management of dental secondary caries.
Author Contributions

Author 1 (Madhura Deshmukh), carried out the study by collecting data and drafted the manuscript after performing the necessary statistical analysis. Author 2 (Dr. Subhabrata Maiti) aided in conception of the topic, has participated in the study design, statistical analysis and has supervised the preparation of the manuscript.

Conflict Of Interest

The authors deny any conflicts of interest related to this study.

Acknowledgement

This research was done under the supervision of the Department of Research of Saveetha Dental College and Hospitals. We sincerely show gratitude to the corresponding guides who provided insight and expertise that greatly assisted the research.

REFERENCES

Mood/publication/265383299_The_Inhibitory_Effect_of_Ajowan_Essential_Oil_on_Bacterial_Growth/links/540c5e1b0c2f2b29a377e50/The-Inhibitory-Effect-of-Ajowan-Essential-Oil-on-Bacterial-Growth.pdf


Table 1: Mean values for Antibacterial activity of Ajwain extract in Streptococcus Mutans and Candida Albicans.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Mean ± SD</th>
<th>d</th>
<th>SE</th>
<th>95%CI</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>SE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus Mutans</td>
<td></td>
<td></td>
<td>SE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incorporated</td>
<td>15.0±2.0</td>
<td>4</td>
<td>1.8</td>
<td>-17.81</td>
<td>-6.82</td>
<td>0.002*</td>
</tr>
<tr>
<td>Coated</td>
<td>27.66±2.51</td>
<td>4</td>
<td>2.0</td>
<td>-25.37</td>
<td>-9.57</td>
<td>0.001*</td>
</tr>
<tr>
<td>Candida Albicans</td>
<td></td>
<td></td>
<td>SE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incorporated</td>
<td>15.66±2.51</td>
<td>4</td>
<td>2.0</td>
<td>-25.37</td>
<td>-9.57</td>
<td>0.001*</td>
</tr>
<tr>
<td>Coated</td>
<td>35.33±2.51</td>
<td>4</td>
<td>2.0</td>
<td>-25.37</td>
<td>-9.57</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Fig 1: A) 5gms of Ajwain seeds were weighed B) Extraction of seeds with 100ml of distilled water C) Solution obtained was re-heated to reduce the extract to 10ml D) 10ml extract stored in sterile test-tube.
Madhura Deshmukh et al: Incorporation Of Ajwain Extract In Provisional Luting Cement For Long Term Inhibition Of Bacterial Infiltration

Fig 2: Zone of inhibition for Streptococcus Mutans and Candida Albicans microorganisms.

Fig 3: Bar represents the anti-bacterial activity of ajwain extract against two microorganisms. Microbial activity of Streptococcus Mutans for Group 1 & 2 had mean value 15.0±2.0 and 27.66±2.51 respectively, p-value 0.002* which shows statistical significance. Microbial activity of Candida Albicans for Group 1 & 2 had mean value 15.66±2.51 and 35.33±2.51 respectively, p-value 0.001* which shows statistical significance.