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Assessment of Concentration of Licorice (Mulethi) Crystals and Ajwain Flower Extract Crystals to Be Used as a Chairside Herbal Disinfectant for Chairside Sterilisation of Orthodontic Wires: In Vitro Study.

[1]Sejul K. Jaiswal, [2]Varunjeet Chaudhary, [3]Johar Rajvinder Singh, [4]Utkarsha Mahorkar, [5]Surabhi Kapgate, [6]Akshata Dand, [7]Madhur Gogulwar, [8]Mayur Pawar, [9]Bhoomika Kulkarni, [10]Ajita Mehendale

1 Postgraduate Student, Department of Orthodontics and Dentofacial Orthopedics, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur

2 Professor, Department of Orthodontics and Dentofacial Orthopedics, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur

3 HOD, Department of Orthodontics and Dentofacial Orthopedics, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur

4 Postgraduate Student, Department of Orthodontics and Dentofacial Orthopedics, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur

5 Postgraduate Student, Department of Orthodontics and Dentofacial Orthopedics, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur

6 Postgraduate Student, Department of Orthodontics and Dentofacial Orthopedics, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur

7 Postgraduate Student, Department of Orthodontics and Dentofacial Orthopedics, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur

8 Postgraduate Student, Department of Oral and Maxillofacial Surgery, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur

9 Postgraduate Student, Department of Paediatric and Preventive Dentistry, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur

10 Postgraduate Student, Department of Oral Medicine and Radiology, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur

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KEYWORDS

Antimicrobial, Herbal Disinfectant, Licorice, Mulethi,

ABSTRACT:

Introduction: The use of fixed orthodontic appliances increases the risk of gingival inflammation and microbial contamination due to biofilm accumulation. Preformed NiTi or stainless-steel wires, which are often handled prior to sterilisation, are susceptible to contamination. While chemical disinfectants like chlorhexidine are effective, they have notable side effects such as staining. Due to increasing resistance of pathogens such as *E. coli* and *S. aureus* and the limitations of conventional agents, this study aims to assess the antimicrobial efficacy of Licorice (Mulethi) and Ajwain extracts at various concentrations for disinfecting orthodontic wires.

Objectives: The study aimed to formulate and evaluate herbal disinfectants containing *Trachyspermum ammi* and *Glycyrrhiza glabra* at different concentrations for the effective disinfection of preformed NiTi archwires.

Methods: Herbal formulations of 5%, 10%, and 20% concentration were prepared using standardized Licorice and Ajwain extracts. Their efficacy was tested on *Streptococcus mutans*, *Staphylococcus aureus, and Escherichia coli* using Mueller-Hinton agar and orthodontic wires. Control was 70% alcohol.

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Results: Among all formulations tested, the 20% concentration demonstrated the highest antimicrobial efficacy, producing the largest zones of inhibition. Statistically significant differences were observed between the treated groups and the control ($p < 0.05$).

Conclusions: Herbal formulations demonstrated potential as effective alternatives for chairside disinfection of orthodontic wires.

1. Introduction

Utilisation of fixed orthodontic apparatuses regularly results in expanding levels of periodontal pathogens in supragingival and subgingival biofilms, causing gingival irritation amid orthodontic treatment. (1) The utilisation of orthodontic apparatuses may increase the chance of creating dental caries and periodontal infections. The advancement of neurotic changes comes about from the disruption of homeostasis of the oral microbiome, and happens beneath the impact of numerous endogenous and outside components, including both the microorganisms and the immunological reaction of the human body. (2) Research shows that many orthodontic professionals use NiTi or S.S. preformed wires directly, which increases the chance of contamination from gloves, dealing with, and/or intentional storage (3). To keep things clean and germ-free during orthodontic treatment, it is very important to quickly clean NiTi and SS wires, as well as handle them using clean tweezers. Different chemicals like ethyl alcohol, paraformaldehyde, formoterol, glutaraldehyde, polyvinylpyrrolidone iodine, quaternary ammonium compounds, and hydrogen peroxide are often used to clean and kill germs. (3) Lucas et al. also isolated aerobic and anaerobic microorganisms from the blood samples of patients undergoing orthodontic treatment, specifically during the placement of separators. (4) Simply rinsing appliances under running tap water is ineffective for proper disinfection. Therefore, the use of an adjunctive disinfectant is essential to minimise the adverse effects of contaminated appliances. Currently, a wide variety of chemical and natural disinfecting agents are available in the market, among which chlorhexidine is considered the gold standard. It exhibits both bacteriostatic and bactericidal properties, depending on the concentration used. However, despite its effectiveness, chlorhexidine is associated with several undesirable side effects, the most common being staining of natural teeth, prostheses, and acrylic appliances encountered in routine dental practice. (5)

Escherichia coli (E. coli) and *Staphylococcus aureus (S. aureus)* are opportunistic pathogens known to cause serious and potentially life-threatening infections, particularly in immunocompromised individuals. The Gram-positive bacterium S. aureus is commonly associated with postoperative wound infections, toxic shock syndrome, and food poisoning. *E. coli*, a Gram-negative bacterium normally residing in the human gastrointestinal tract, can lead to infections such as lower urinary tract infections, cholecystitis, and septicemia. Several studies have reported increasing resistance of both *S. aureus* and *E. coli* to commonly used antibiotics, highlighting the urgent need for alternative antimicrobial strategies. (6) A review of the literature has shown that essential oils (EOs) derived from various species of peppermint possess notable antimicrobial properties against a range of plant pathogens, along with insecticidal activity on stored products. (7) Building upon this concept, the present study aims to evaluate the antimicrobial activity of Licorice (Mulethi) and Ajwain crystals, and to assess the efficacy of different concentrations of these herbal extracts in disinfecting preformed orthodontic wires.

2. Objectives

This study aimed to develop an herbal disinfectant of different concentrations and to compare and evaluate the efficacy of this formulation comprising Ajwain flower (Trachyspermum ammi) and Licorice (Glycyrrhiza glabra) for effective disinfection of preformed NiTi arch wire.

3. Methods

a. Collection and preparation of Ajwain flower and Licorice Crystals:

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Fresh Ajwain flowers and Licorice crystals were obtained from reliable sources.

Figure - 1

b. Formulation development:

The extracts were mixed in varying ratios to determine the optimal combination for maximum antimicrobial activity.

A total of 4 formulations were prepared-

1. 70% alcohol used as the Standard formulation

Figure 2- Standard Sample/ Sample 1

2. 5% concentration solution- in which 2.5 g of Ajwain and 2.5 g of Licorice (Mulethi) crystals were mixed with 10 ml of H2O

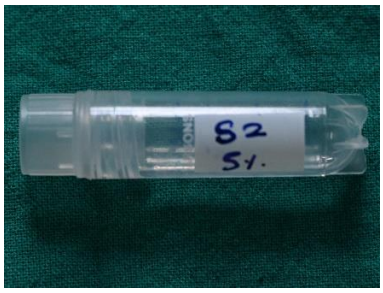

Figure 3. Sample 2

3. 10% concentration solution – in which 5 g of Ajwain and 5 g of Licorice (Mulethi) crystals were mixed with 10 ml of H_2O.

Figure 4. Sample 3

4. 20% concentration solution- which contain 10 g of Ajwain and 10 g of Licorice (Mulethi) with 10 ml of H2O.

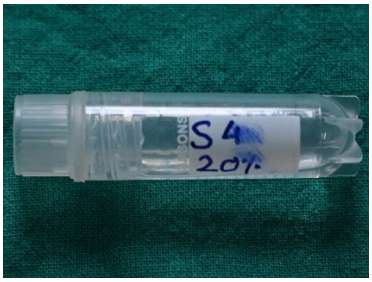

Figure 5. Sample 4

Stabilisers and preservatives were incorporated to improve shelf life.

c. Antimicrobial efficacy testing:

Mueller-Hinton agar medium was used for broth microdilution tests to determine the Minimum Inhibitory Concentration (MIC) and the zone of inhibition against representative dental pathogens (e.g., *Streptococcus mutans, Enterococcus faecalis, Candida albicans*).

d. Compatibility testing:

A piece of unused commercially available preformed NiTi archwire was placed in broth to see the number of bacteria that contaminated the wire and to assess the colonies formed by bacteria.

Unused preformed NiTi archwire was immersed in the herbal disinfectant for a specified duration to observe the disinfectant properties on the instruments.

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e. Comparative analysis:

Comparison of antimicrobial efficacy of the different concentrations of herbal disinfectant with a commercially available preformed NiTi and stainless-steel wire treated with and without disinfectant, and through standard testing protocols, was conducted.

INCLUSION CRITERIA:

1. Unused wires freshly taken from the packet

EXCLUSION CRITERIA:

1. Used or contaminated wire (from the patient's mouth).

A total of 14 Petri plates were cultured with Mueller-Hinton agar broth plates.

12 plates were divided into 3 groups according to bacteria.

Group A – *E. coli*

Group B- *Staphylococcus aureus*

Group C- *Streptococcus mutans*

Each bacterium contains 4 plates, i.e,

4 plates for *E. coli*-

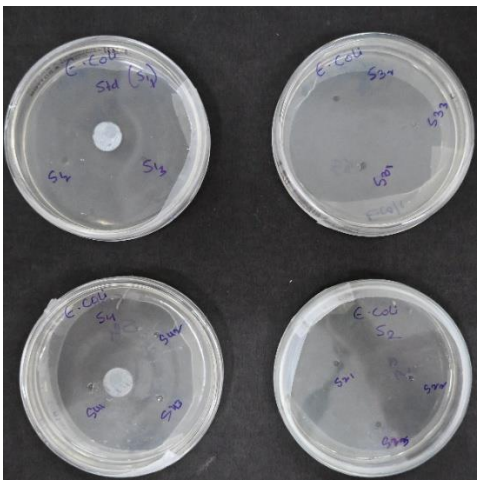

Figure 6- before the growth of the bacterial colony

3 plates for *Staphylococcus aureus*-

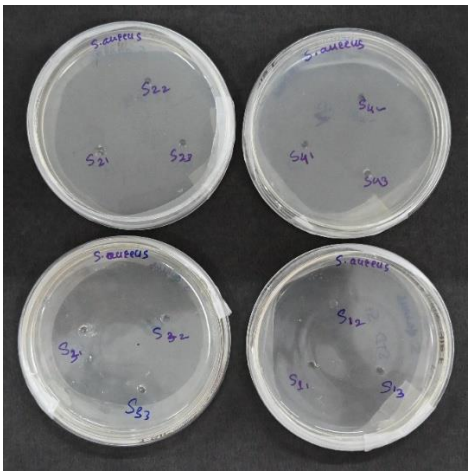

Figure 7- before the growth of the bacterial colony

3 plates for *Streptococcus mutans*-

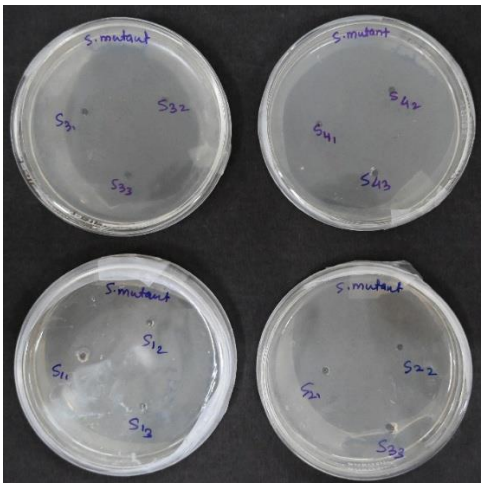

Figure 8- before the growth of a bacterial colony

In all these plates, 3 wells were prepared, which were filled with the same concentration samples along with bacteria inoculation to see the effect of these different concentration samples on the bacterial colony.

In plate 1- standard alcohol

In plate 2 – 5% concentration (i.e sample 2)

In plate 3 – 10 % concentration (i.e sample 3)

In plate 4 – 20 % conc. Solution (i.e sample 4)

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In the remaining two plates, orthodontic wires were placed in the media to evaluate bacterial contamination without and with disinfection.

One plate contained untreated orthodontic wires to serve as a negative control.

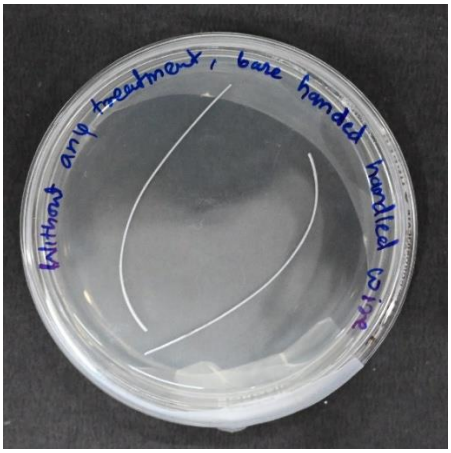


Figure 9

On a plate, 11- 3 pieces of wires were placed with the treatment of different concentrations of the sample or herbal disinfectant.

The wires were dipped into the different concentrations of herbal disinfectant and placed in the Mueller-Hinton agar to see the effect of disinfectant on the wire.

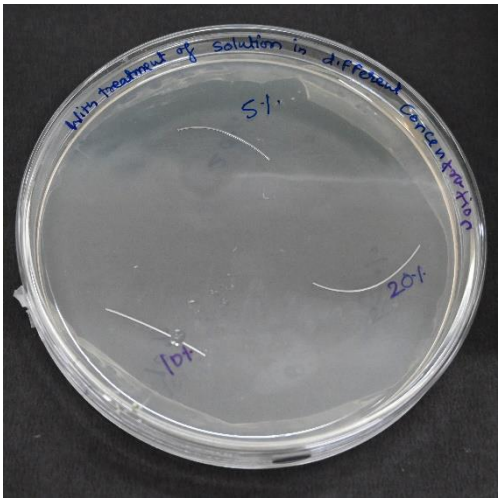


Figure-10

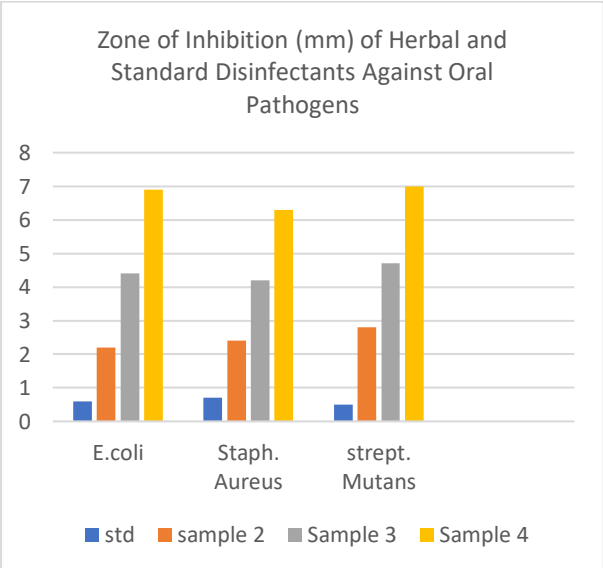
All these plates were placed in an incubator for 24 hrs for the formation of colonies and to see the effect of different concentrations of herbal disinfectant samples against these bacteria.

Statistical Analysis-

Descriptive statistics, including mean, standard deviation (SD), and range, were used to summarise the antimicrobial properties of the herbal extracts. Statistical analysis was performed using STATA (version 10.1, Stata Corp. LLC, College Station, TX, USA). **Two-way ANOVA** was conducted to assess the effects of herbal extract concentration and bacterial strain on antimicrobial efficacy. **One-way ANOVA** with **post hoc Bonferroni's test** was used to compare mean inhibition zone diameters between groups. The **χ^2 test** was applied to evaluate the contamination rates of orthodontic wires and the effectiveness of the herbal extracts in decontaminating them. A **p-value < 0.05** was considered statistically significant.

4. Results

All herbal formulations exhibited antimicrobial activity. The 20% concentration showed the largest zones of inhibition for all bacteria tested, followed by 10% and 5% concentrations. Herbal-treated wires displayed significantly fewer colonies compared to untreated wires ($p<0.05$). The performance of the 20% formulation was comparable to that of the standard alcohol control.



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5. Discussion

The results confirmed that herbal formulations of Licorice and Ajwain are effective against major oral pathogens. The 20% solution had superior efficacy. These herbal alternatives support biocompatibility and green dentistry practices.

The higher efficacy of the 20% formulation suggests a concentration-dependent antimicrobial effect of the herbal extracts. This aligns with previously reported properties of Glycyrrhiza glabra and Trachyspermum ammi, both known for their active phytochemicals like glycyrrhizin and thymol, which exhibit strong antibacterial activity. The current study reaffirms that these components are particularly effective against Gram-positive organisms such as *Staphylococcus* and *Streptococcus mutans*, as well as Gram-negative *E. coli*.

Interestingly, the herbal formulations demonstrated antimicrobial performance comparable to that of 70% alcohol, a standard disinfectant, without the associated drawbacks like staining, mucosal irritation, or cytotoxicity. This is especially relevant in clinical orthodontics, where repeated exposure to chemical disinfectants may lead to material degradation and patient discomfort.

Additionally, this study offers a sustainable, cost-effective alternative that supports the global movement toward green dentistry. The ease of preparation and natural origin of the ingredients make these formulations suitable for routine chairside use. Further investigations, including clinical trials and stability analysis, will be needed to validate long-term effectiveness and safety in real-world orthodontic practice.

6. Conclusion

In conclusion, the findings support the use of 20% Licorice and Ajwain formulation as an effective, natural alternative to conventional disinfectants for chairside sterilisation of orthodontic wires.

These findings align with the principles of green dentistry and promote patient safety in clinical orthodontic practice.

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