ORIGINAL ARTICLE

Comparison of Antimicrobial Efficacy of Natural Extracts as a Disinfectant for Removable Orthodontic Appliances: An *Ex Vivo* Study

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Abstract

Aim: The purpose of the study is to formulate a standard protocol for cleaning and disinfecting removable appliances by evaluating the antimicrobial efficacy of three natural extracts.

Materials and methods: Fifty patients with removable orthodontic appliances were selected and divided into five groups [chlorhexidine 0.2% (positive control), extracts of *neem*, *katha*, cinnamon, and normal saline (negative control)] with 10 samples each. Saliva sample from the appliance was collected using a sterile cotton swab and cultured. Agar diffusion method was used and zones of inhibition were measured.

Statistical analysis: The obtained data were subjected to statistical analysis by the Kruskal–Wallis ANOVA test and the Mann–Whitney *U* test.

Results: The *neem* extract had the highest zone of inhibition measuring 14.95 mm (mean) followed by *katha* extract with the value of 13.85 mm which was found to be comparable to chlorhexidine with 15.85 mm.

Conclusion: Thus, *neem* and *katha* extracts can be used to disinfect removable appliances because of their good antimicrobial activity.

Clinical significance: By following these simple cleaning protocols, it is possible to avoid numerous health hazards caused by unclean removable appliances in children.

Keywords: Appliances, Disinfection, Natural extracts, Removable orthodontics.

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Introduction

Removable orthodontic appliances are in use since the early 1900s, pioneered by George Crozat in the US, and were then modified according to the treatment needs. Removable orthodontic appliances have their own benefits and limitations. They are affordable, easy to use, and easy to adjust and the treatment time is less when compared to the fixed orthodontic appliances. But still, there are certain limitations like patient compliance and it can be used only for minor teeth movements which require only tipping forces. In young children and adolescents, hence removable orthodontic appliances are preferred over fixed, for correction of such minor malocclusions.

The major problem is encountered when it comes to the cleaning and disinfection of these appliances. The awareness about the maintenance of clean appliances and the harmful effects of the unclean appliance is very minimal among the Indian population.

Appliances containing debris and stains will irritate the tissues and the subsequent tissue response in the oral cavity. Oral appliances can harbor an entity of microorganisms which may serve as reservoirs for disseminated infections with gastrointestinal and pleuropulmonary involvement.

Cleaning of the appliance in the running tap water will not be of any use. Hence, the use of a disinfectant in adjunct is necessary to minimize the adverse effects of unclean appliances.

At present, there are many chemicals as well as natural disinfecting agents are available in the market, of which chlorhexidine is considered as the gold standard. It is both bacteriostatic and bactericidal in nature when used in low and high concentrations, respectively. Still, there are considerable adverse effects of this chemical of which staining of natural and artificial teeth and acrylic plates (if any) is the most frequently encountered in dental practice.

Azadirachta indica, commonly known as *neem*, is one such medicinal plant that can be used for a wide range of diseases. More than 140 compounds have been isolated from parts of *Neem*. All the parts of the *neem* tree—leaves, flowers, seeds, fruits, roots, and bark have been used for the treatment of inflammation, infections, fever, skin diseases, and dental disorders. *Neem* leaf and its constituents have been found to exhibit immune-modulatory, antiinflammatory, antihyperglycemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, and anticarcinogenic properties.

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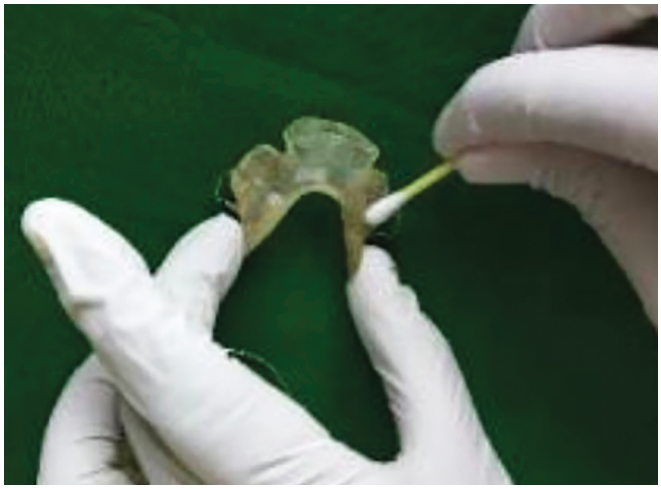

Fig. 1: Collection of a salivary swab

Fig. 2: Microbial analysis

Acacia catechu, commonly known as *katha* is known to be a strong antioxidant, astringent, anti-inflammatory, antibacterial, and antifungal agent used for many medicinal purposes. It is used to treat sore throat and diarrhea and high blood pressure, dysentery, colitis, gastric problems, bronchial asthma, cough, and leprosy. And also used as a mouthwash for gingival infections.

Cinnamon is a medicinal plant which is in use in various field of which dental is one among them.

Materials and Methods

The study population consisted of 50 children using removable orthodontic appliances. They were then divided into five groups with each group having ten participants. Five components used in the study were: Chlorhexidine as a positive control, *neem* extract, *katha* extract, cinnamon extract, and normal saline as a negative control.

The selection of the participants was based on the following criteria.

Inclusion Criteria

- Children using removable orthodontic appliances.
- Children with mixed dentition.

Exclusion Criteria

- Children who do not visit recall appointments regularly.
- Children with only primary or permanent dentition.

Collection of the Sample

Participants were selected and salivary swabs were collected from the palatal surface of the appliance according to a 2 × 2 cm template (Fig. 1). The swabs were then placed in a test tube containing thioglycollate broth and then vortexed for 1 minute which was then subjected to microbial culture.

Preparation of Ethanolic Extracts of *Neem*, *Katha*, and Cinnamon

Ten grams of dried leaf powder of *neem*, *katha*, and cinnamon were taken in a separate container. To this, 100 mL of ethanol was added to get a 10% extract. The powder and ethanol were ground in a mortar and pestle and kept for 24 hours with periodic shaking and then filtered through Whatman no 1 filter paper and the filtrate was then collected. This procedure was repeated thrice and the collected filtrates were pooled. The filtrate was evaporated to dryness by leaving it inside the incubator at 37°C for 2 hours. The residues obtained were stored at 4°C until testing.

Antimicrobial Susceptibility Testing

Brain–heart infusion agar plates were used. The agar plates were inoculated with the sample and allowed to set for 30 minutes. Five wells were then prepared in the agar plates using the template. The agents were then inoculated in the agar plates using micropipettes. These plates were kept inside the incubator at 37°C for 24 hours and then checked for their respective zones of inhibition. A uniform circular zone of inhibition will be formed around all five wells. The diameter of all the zones was then measured to the nearest whole millimeter with a ruler. The net results were recorded and the pictures of the plates were taken using a digital camera (Fig. 2).

Statistical Analysis

The results were analyzed using SPSS version 17 software. Kruskal–Wallis ANOVA test followed by the Mann–Whitney *U* test was used to compare the zones of inhibition between the five agents. A difference was considered to be of statistical significance if the *p* value was <0.05 (Fig. 3).

Results

A total of 50 participants were in the study group. Table 1 shows the mean zones of inhibition of all the groups with *neem* being the closest to the positive control (chlorhexidine) followed by *katha* and then cinnamon extracts.

Table 2 shows the Kruskal–Wallis ANOVA test, with *p* value < 0.001 proving the statistical significance of the study.

Table 3 shows the Mann–Whitney *U* test for comparison between the groups with the *p* value <0.05.

Discussion

The main purpose of this study is to formulate a standard protocol for cleaning and disinfecting the removable orthodontic appliances used by children and adolescents using various natural extracts as the disinfecting agents.

Oral appliances can harbor an entity of microorganisms which may serve as reservoirs for disseminated infections with

gastrointestinal and pleuropulmonary involvement. For this reason, disinfection plays a pivotal role.

As discussed earlier, even though chlorhexidine is considered the gold standard, it still has its own drawbacks. Hence to overcome those drawbacks, natural extracts were chosen in this study.

Through this study, we are trying to educate the patients as well as their parents about general oral health and the maintenance of these removable appliances.

There are various studies done to show the antimicrobial efficacy of chlorhexidine.

A study conducted by Tereza et al. in 2010 showed that chlorhexidine (2%) gluconate had the highest antimicrobial activity when compared with 1% sodium hypochlorite and paramonochlorophenol combined with furacin against *S. aureus*, *C. albicans*, *E. faecalis*, and *P. aeruginosa*.[1]

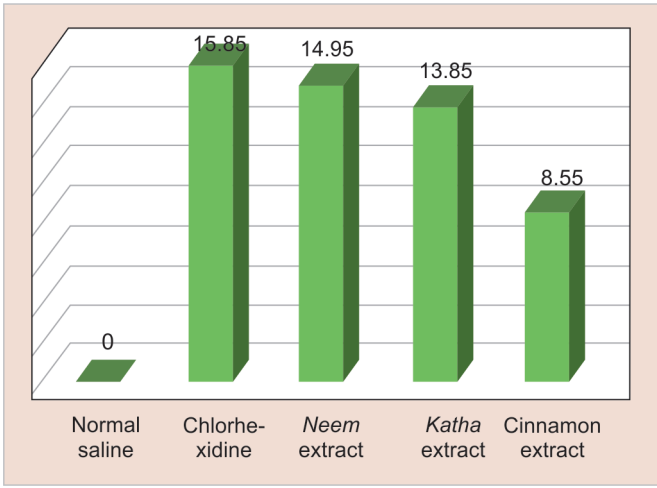

Fig. 3: Mean zones of inhibition

Despite its good antimicrobial activity, its widespread and prolonged use is limited due to its local side effects in which discoloration/staining of natural and artificial teeth (and acrylic if any) is the most common. A dark yellow or brown stain is often seen only after a few days of use. Other side effects include transient impairment of taste sensation or taste perturbation where salt taste appears to be affected parotid swelling is a rare adverse effect. Occasionally reported are cases of burning sensation and painful desquamative lesions. It also exhibits a bitter taste which is difficult to mask completely.[2]

A study done by Francine et al. in 2015 showed that ethanolic extract of *neem* leaves and bark had more efficient antimicrobial

Table 3: Mann–Whitney *U* test

Reference group	*Comparison group*	*Mean difference*	*p*
	Group II chlorhexidine	−15.850	<0.001
Group I normal saline	Group III *neem* extract	−14.950	<0.001
	Group IV *katha* extract	−13.850	<0.001
	Group V cinnamon extract	−8.550	<0.001
	Group III *neem* extract	0.900	<0.001
Group II chlorhexidine	Group IV *katha* extract	2.000	<0.001
	Group V cinnamon extract	7.300	<0.001
	Group IV *katha* extract	1.100	0.001
Group III *neem* extract	Group V cinnamon extract	6.400	<0.001
Group IV *katha* extract	Group V cinnamon extract	5.300	<0.001

Table 1: Mean zones of inhibition of normal saline, *neem, katha*, and cinnamon extracts

Sample	*Group I–Normal saline*	*Group II–Chlorhexidine*	*Group III–Neem extract*	*Group IV–Katha extract*	*Group V–Cinnamon extract*
Sample 1	0 mm	15.5 mm	15 mm	13 mm	9 mm
Sample 2	0 mm	16 mm	15.5 mm	13 mm	8.5 mm
Sample 3	0 mm	16 mm	14.5 mm	14 mm	9 mm
Sample 4	0 mm	16 mm	15 mm	13 mm	9.5 mm
Sample 5	0 mm	15.5 mm	15 mm	14 mm	8 mm
Sample 6	0 mm	16 mm	14 mm	15 mm	8 mm
Sample 7	0 mm	15.5 mm	15 mm	14 mm	8 mm
Sample 8	0 mm	16 mm	15.5 mm	14.5 mm	8.5 mm
Sample 9	0 mm	15.5 mm	15 mm	14 mm	8 mm
Sample 10	0 mm	15.85 mm	14.95 mm	13.85 mm	8.55 mm
Mean	0 mm	15.85 mm	14.95 mm	13.85 mm	8.55 mm

Table 2: Kruskal–Wallis ANOVA test

		Kruskal–Wallis test				
		Zone of inhibition				
		Mean	*SD*	*Chi-square*	*df*	*p*
Group I normal saline	J0	0.000	0.000	46.634	4	<0.001
Group II chlorhexidine	J0	15.850	0.337			
Group III *neem* extract	J0	14.950	0.437			
Group IV *katha* extract	J0	13.850	0.668			
Group V cinnamon extract	J0	8.550	0.550			

activity when compared to the aqueous extract against *S. aureus* but *E. coli* did not respond to either aqueous or ethanolic extract.[3]

A study conducted by Parashar et al. in 2018 showed that the combined antimicrobial effect of *neem* and tantani showed excellent antimicrobial activity against *E. coli*, *P. aeruginosa*, and *B. subtilis* than their individual extracts.[4]

A study was conducted by Yuvaneswaran et al. in 2015 to show the cytotoxicity and antimicrobial properties of *neem* leaf extracts. Acetone extract exhibited stronger inhibition against gram-negative organisms and had the highest cytotoxicity and chloroform extract exhibited stronger inhibition against gram-positive organisms.[5]

A study was done by Vibha et al. in 2013 to compare the antimicrobial activity of *neem*, propolis, turmeric, liquorice, and sodium hypochlorite as root canal irrigants against *E. faecalis* and *C. albicans* and *neem* had the highest activity.[6]

In a study conducted by Wasudeo et al. in 2013, *neem* showed the highest antibacterial efficacy against *E. faecalis* when compared to grape seed extract and 3% sodium hypochlorite.[7]

A study conducted by Biswas et al. in 2014 showed that a herbal mouthwash containing *neem*, clove, triphala, tulsi, celery, licorice, oak tree, *katha*, and spearmint produced equivalent antibacterial activity when compared to chlorhexidine mouthwash.[8]

In a study done by Lakshmi et al. in 2011, it was found that ethanolic extract of *Acacia catechu* willd had strong antibacterial activity against gram-negative bacilli and gram-positive cocci.[9]

A study done by Syahdiana et al. in 2018 showed various concentrations (6.25, 12.5, and 25%) of the cinnamon extract showed excellent antimicrobial effect by inhibiting almost six streptococci species and hence can be used as a mouthwash.[10]

A study was conducted by Rita et al. in Brazil in 2016 to evaluate the frequency of cleaning and the agents used for cleaning the removable appliances among adolescent children and the recommendations given by the dentist. It was found that brushing with toothpaste was the most commonly followed method, followed by brushing with soap, mouthwash, corega tabs, and just water. And the majority cleaned the appliances only once in a day.[11]

So from this study, we hereby found *neem* and *katha* to be effective disinfectants as comparable to chlorhexidine and hence can be used for cleaning the removable orthodontic appliances.

Studies are yet to be done *in vivo*, with a bigger sample size by giving the solutions to the patients and check their efficacy clinically.

Conclusion

Hence, it is very crucial to clean the removable orthodontic appliances every day. Natural agents have proven to be a good disinfectant with minimal adverse effects when compared to chemical agents.

Clinical Significance

By following these simple cleaning protocols, it is possible to avoid numerous health hazards caused by unclean removable appliances in children.

References

1. Semenoff TDV, Semenoff-Segundo A, Henrique Borges A, et al. Antimicrobial activity of 2% chlorhexidine gluconate, 1% sodium hypochlorite and paramonochlorophenol combined with furacin against *S. aureus, C. albicans, E. faecalis* and *P. aureginosa*. Rev Odonto ciênc 2010;25(2):174–177.
2. Mathur S, Mathur T, Srivastava R, et al. Chlorhexidine: the gold standard in chemical plaque control. Nat J Physiol, Pharm Pharmacol 2011;1(2):45–50.
3. Francine U, Jeannette U, Pierre. RJ. Assessment of antibacterial activity of neem plant (*Azadirachta indica*) on *Staphylococcus aureus* and *Escherichia coli.* J Med Plants Stud 2015;3(4):85–91.
4. Parashar G, Sutar N, Sanap S. Antibacterial activity of mixture of leaf extracts of neem (*Azadirachta indica* Linn.) and Tantani (*Lantana camara)* Int J Pharm Sci Res 2018;9(6):2545–2549.
5. Krishnan Y, Wong NK. Cytotoxicity and antimicrobial properties of neem (*Azadirachta indica*) leaf extracts. Int J Pharm Sci 2015;7(2):179–182.
6. Hegde V, Kesaria DP. Comparative evaluation of antimicrobial activity of neem, propolis, turmeric, liquorice and sodium hypochlorite as root canal irrigants against *E. faecalis* and *C. albicans* - an in vitro study. J Endodontol 2013;25(2):38–45.
7. Ghonmode WN, Balsaraf OD, Tambe VH, et al. Comparison of the antibacterial efficiency of neem leaf extracts, grape seed extracts and 3% sodium hypochlorite against *E. feacalis* – an in vitro study. J Int Oral Health 2013;5(6):61–66.
8. Biswas G, Anup N, Acharya S, et al. Evaluation of the efficacy of 0.2% chlorhexidine versus herbal oral rinse on plaque induced gingivitis - a randomized clinical trail. IOSR J Nurs Health Sc 2014;3(2V):58–63. DOI: 10.9790/1959-03255863.
9. Lakshmi T, Geetha RV, Roy A. In vitro antibacterial activity of ethanolic bark extract of acacia catechu willd against enteric pathogens. Int J Drug Dev Res 2011;3(3):328–334.
10. Waty S, Suryanto D, Yurnaliza. Antibacterial activity of cinnamon ethanol extract (*Cinnamomum burmannii*) and its application as a mouthwash to inhibit streptococcus growth. IOP Conf. Series: Earth and Environmental Science 130 (2018) 012049.
11. Souza Lamas RR, Suca Salas MM, Cenci TP, et al. Removable orthodontic appliances: frequency and cleaning agents used by students and recommended by dentists. Braz J Oral Sci 2016;15(1):21–26. DOI: 10.20396/bjos.v15i1.8647093.