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Comparative Evaluation of Antimicrobial Efficacy of Neem and Ginger, To Prove Its Effectiveness in Decontaminating "Gutta Percha Cones" - An In Vitro Study

Dr. Abitha banu.M., Dr. Sebeena Mathew, Dr. Karthick.K, Dr. Deepa.T., Dr.Boopathi.T, Dr. Poojitha.V., Dr.Loganathan.M.

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Aim:

To compare and evaluate the antimicrobial efficacy of neem, ginger, neem plus ginger and 5.25% sodium hypochlorite and to determine its effectiveness in decontaminating "gutta percha cones"

Materials and Methods:

A concentrated extract of neem, ginger and neem plus ginger are used to check for the antimicrobial efficacy using the agar well diffusion method. Presence of zones of diffusion were identified against 3 common gutta-percha contaminants namely E.coli, E.faecalis and Staph aureus. New gutta-percha cones, freshly taken out of the packet using sterile tweezers were then decontaminated for 1minute using 6%neem, 6%ginger and 6% neem plus ginger and 5.25% sodium hypochlorite separately. Then, it was placed in a nutrient broth to check for the presence of turbidity.

Results: The zones of inhibition were formed around both neem and ginger natural extract, in which neem plus ginger group showed almost equivalent antimicrobial efficacy in comparison to 5.25% Sodium hypochlorite. The broth remained clear even after 24 hours of incubation.

Conclusion: We observed in this study that *neem plus ginger extract* is indeed effective as a GP decontaminant and it holds a promising future as a medium for storage of GP cones.

Keywords: *Neem and ginger*; decontamination; gutta percha cones

Introduction:

The success of endodontic therapy lies in maintaining an aseptic condition right from the beginning of access opening till restoring the tooth structure to its original form and function. Elimination of micro-organisms from the root canal system plays a vital role. Gutta percha (GP) the most commonly used obturating material is obtained from a dried coagulated extract of plants named palaquiam of the blanco genus of sapotaceae family, and was introduced to dentistry in 1847 by Edwin Truman.[1,2] It still continues to maintain its position as an important dental material and has emerged as the prime root canal filling material.

The SS White Company began marketing GP points for dental use in 1887. GP supplied commercially is not usually sterilized and it should be decontaminated before obturation. GP carries risk of physical deformation so it cannot be sterilized by moist or dry heat.

However, chair side disinfection prior to obturation should be performed prior to obturation. Many chemicals such as, hydrogen peroxide, chlorhexidine, ethyl alcohol, polyvinyl pyrolidone iodine, quaternary ammonium compounds have been tried for GP decontamination. Recently, the use of electron beam sterilization has also been tried. However, none of these methods have been proven as fully effective. The recommended method for decontamination of GP points consists of treating the cones using 1% Sodium hypochlorite for 1 minute (Milton's solution), or 0.5% Sodium hypochlorite for 5 minutes (Dakin's solution).[3] Here, there is a risk of Sodium hypochlorite causing crystal deposition within the canals which can impede the obturation and cannot be ignored. The purpose of this study was to evaluate the effectiveness of a herbal alternative, neem, ginger, neem plus ginger and and to determine its effectiveness in decontaminating "gutta percha cones" in comparison with 5.25% sodium hypochlorite.

For thousands of years the beneficial properties of Neem (Azadirachta indica A.Juss) have been recognized in the Indian tradition. Each part of the neem tree has some medicinal property. Biswas et al (2002) [4] have recently reviewed the biological activities of some of the neem compounds, pharmacological actions of the neem extracts, performed a clinical study and studied on the medicinal applications of neem along with their safety evaluation. Biological activity of Neem compounds are wide. It has Anti-inflammatory Antiarthritic, Antipyretic, Antifungal and Antibacterial, Antimalarial properties.It acts as a spermicidal, Diuretic and has Immuno modulatory effects.[5]

Ginger belongs to the Zingiberaceae family, Gingerol is the main bioactive compound in ginger, responsible for much of its medicinal properties. It has powerful anti-inflammatory and antioxidant effects. [6] In fact, ginger extract can inhibit the growth of many different types of bacteria. [7] It is very effective against the oral bacteria linked to inflammatory diseases in the gums, such as gingivitis and periodontitis. [8]

Materials and Methods:

Test Organisms:

Reference strains of three most common GP contaminants, Eschericia coli, Enterococcus faecalis and Staphylococcus aureus were used in this study.

Preparation of the Extract:

The neem leaves were washed with distilled water. Fresh ginger pieces were collected and cut into small pieces. Both neem leaves and freshly cut ginger pieces were dried under the shade and then powdered. Then the powder was macerated with 100% ethanol followed by filtration. An extract was obtained by dissolving 20 grams of the powder in 200 ml of ethanol. The contents were then filtered through Whatmann filter paper no.1, and the filtrate was evaporated for dryness.

Evaluation of antimicrobial activity of Neem, Ginger and Neem plus Ginger:

The antibacterial activity of the extract was tested using Agar well diffusion technique. The reference strains were cultured overnight in nutrient broth, and the culture was streaked on a plate of MHA (Mueller Hinton Agar) (n=15). 5 MHA plates were prepared for each organism. Five wells measuring 6 mm × 6 mm were made with the help of a template on the surface of the agar plate. About 0.1 ml of the extract was delivered into the well using a micropipette. First well with a neem extract, second well with a ginger extract, third well with a neem plus ginger extract, other two (fourth and fifth) wells were filled with 5.25% of sodium hypochlorite and 0.9% normal saline as positive and negative controls, respectively. They were then incubated at 37°C for 24 hours, and closely monitored for the development of clear zones around the extracts. The antibacterial activity was assessed by the diameter of the inhibition zone. A clear zone of inhibition was obtained against all the three organisms.

Gutta Percha Decontamination:

A new pack of Dia-ProT^{plus} (Diadent Europe.B. V,Almere, Netherlands) F2 Size Protaper gutta percha points were used for the procedure. The pack was opened under sterile conditions and 10 points were taken out using a sterile tweezer. The points were then placed inside the freshly prepared nutrient broth, and incubated for 24 hours. Simultaneously, 10 new GP cones were removed,

and decontaminated for one minute in neem extract, ginger extract, neem plus ginger extract, 5.25% sodium hypochlorite and normal saline separately. The cones were then removed from the extracts and cleaned. To make it free from residual extracts sterile gauze was used and then incubated in Nutrient broth for 24 hours. Both the tubes were then closely monitored for the development of turbidity.

Results:

The antimicrobial efficacy was assessed by the presence of zones of inhibition [Figure 1]. Inhibition zones were formed according to the extract used against Escherichia coli, Enterococcus faecalis and Staphylococcus aureus respectively. Zone of inhibition was evident with all extracts that are used except with the negative control normal saline (no zone formation). The zone of inhibition formed with neem plus ginger group showed almost equivalent antimicrobial efficacy in comparison to 5.25% Sodium hypochlorite which was used as a positive control [Table 1]. The GP cones which were not decontaminated and directly placed in the broth developed turbidity. The cones decontaminated with Extracts and then placed in the broth remained clear even after 24 hours, indicating the absence of the microbial contaminants. [Figure 2].

Table 1: Zones of inhibition in millimeters (mm) obtained against the test organisms:

	Zones of inhibition				
	Neem extract	Ginger extract	Neem plus Ginger extract	5.25% Sodium hypochlorite	Normal saline
Eschericia coli	9mm	13mm	14mm	15mm	No zone
Enterococcus faecalis	11mm	11mm	18mm	19mm	No zone
Staphylococcus. aureus	10mm	10mm	16mm	17mm	No zone

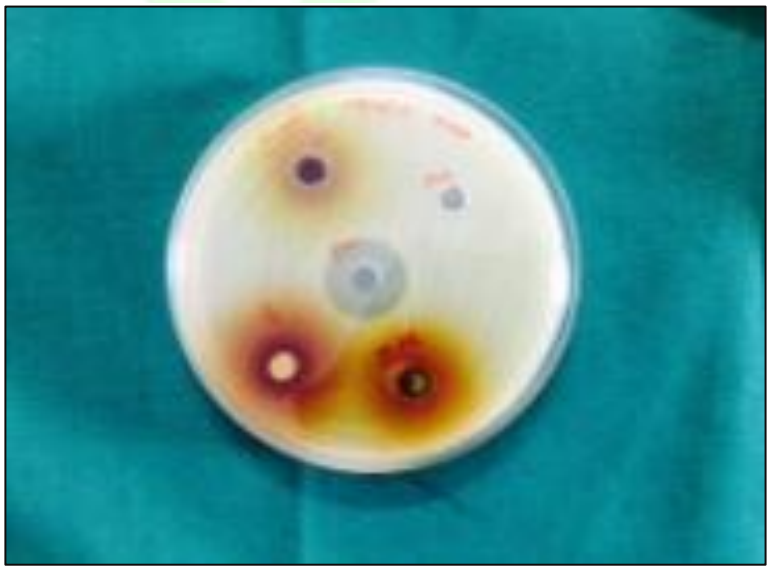

Figure 1: Zone of inhibition by Agar Well Diffusion Method

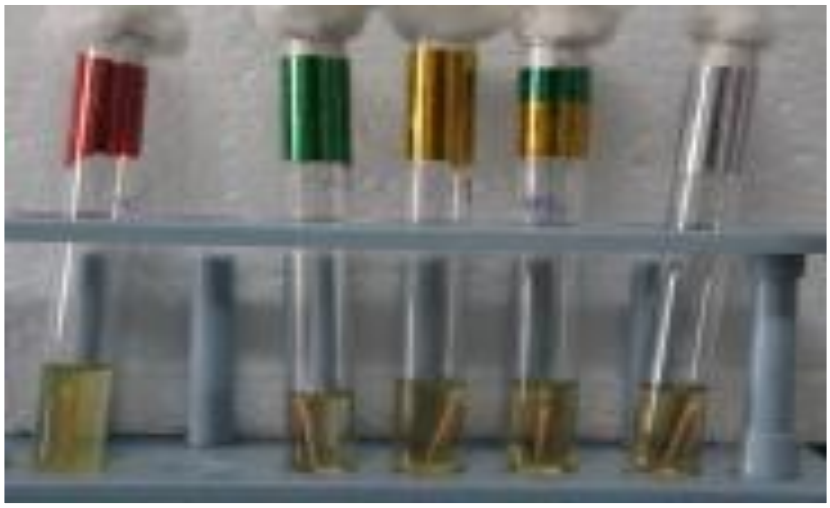

Figure 2: Turbidity test using nutrient broth. Test tube (Red) shows Turbidity- Normal Saline. Test tube (Green) neem extract treated GP, Test tube (Yellow) Ginger extract treated GP, Test tube (Green andYellow) neem plus Ginger extract treated GP and Test tube (Silver) NaOCl treated GP showed no turbidity.

Discussion:

The importance of GP decontamination to prevent any bacterial contamination of the root canal during the obturation procedure is now widely recognized in endodontic practice. Thus, it is imperative to employ a rapid, reliable, inexpensive and effective decontaminant. Glutaraldehyde has been effectively used as a chemosterilizer or a high level disinfectant. Aqueous solutions of 2% glutaraldehyde have a broad spectrum of action and are thus effective against most of the micro-organisms, and have been used effectively for decontaminating endodontic files prior to sterilization in a glass bead sterilizer. However, Boucher found that Bacillus subtilis spores are resistant to treatment with Glutaraldehyde.[9–11] 70% concentrated Ethanol is widely used in dentistry. However, studies indicate that it provides an intermediate level of disinfection, and the surface requiring decontamination requires to be submerged atleast for 10 minutes.[12] 2% Chlorhexidine kills bacteria by disruption of the cell membranes and by inducing precipitation of the cytoplasm. It has however been reported by Sequeira et al that chlorhexidine is ineffective even after 10 minutes of surface exposure and requires much longer durations of contact.[13–15]

Sodium hypochlorite has a strong antibacterial and sporicidal effect, and acts by a mechanism involving the liberation of active chlorine, (a powerful oxidizing agent) which in turn inactivates the bacterial enzymes. Sodium hypochlorite 5.25% has been found to be effective in decontaminating GP cones. However, it is imperative that after disinfection, the GP cone should be rinsed in ethyl alcohol to remove crystallized sodium hypochlorite before obturation as the crystals may impair the hermetic seal.

It is well known that neem leaves (A. indica or AI) have antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antipyretic, and analgesic effects without any side effect. AI has several active constituents like nimbidin, nimbin, nimbolide, gedunin, azadirachtin, mahmoodin, margolone, and cyclictrisulfide which are responsible for its antibacterial action. AI causes maximum reduction in adherence of E. faecalis to dentin. [15] In our study results are in accordance with Vinothkumar et al [15] and Ghonmode et al [16] They found that neem was highly effective against E. faecalis compared to 5.25% sodium hypochlorite and other extracts.

Analyzing the active constituents of ginger, Lantz et al[17] verified that gingerol was capable of significantly inhibiting the production of prostaglandins E_2 induced by LPS from Escherichia coli. The extracts containing mainly gingerol had the capacity of inhibiting COX-2 production.

In this study while comparing zone of inhibition against common GP contaminants, individual neem and ginger extract was comparatively less than that of combined mixture of neem and ginger extract. The combined mixture of neem and ginger extract showed a larger zone of inhibition. This proves its synergistic effect on its combination of neem plus

ginger. Another major advantage is that neem and ginger extract has been found to be effective in decontaminating GP cones within one minute. To substantiate these results, further in depth studies incorporating more isolates from clinical samples are required.

Conclusion:

Within the limits of this study, it can be concluded that neem plus ginger can be used effectively for decontaminating GP cones within a short duration, and holds a promising future as a medium for storage of GP points.

References:

1. Cruse WP, Bellizi R. A historic review of endodontics 1689-1963, Part I. J Endod. 1980; 6:495–9.
2. Jyoti BB. Phytotherapeutics in conservative dentistry and endodontics - a review. J Conserv Dent. 2005; 8:31–9.
3. Cardoso CL, Kotaka CR, Redmerski R, Guilhermetti M, Queiroz AF. Rapid decontamination of gutta percha cones with sodium hypochlorite. J Endod. 2009; 25:498–501.
4. Biswas, Kausik, Ishita Chattopadhyay, Ranajit K. Banerjee and Uday Bandyopadhyay. 2002. Biological activities and medicinal properties of Neem (Azadirachta indica). Current Science 82(11): 1336-1345.
5. D.P. Agrawal (n.d.). "Medical properties of Neem: New Findings".
6. Biological properties of 6-gingerol: a brief review.Wang S, Zhang C, Yang G, Yang Y.2014 Jul;9(7):1027-30.
7. Antibacterial effect of Allium sativum cloves and Zingiber officinal lerhizomes against multiple-drug resistant clinical pathogens. Ponmurugan Karuppiah and Shiyamkumar Rajaram[2] Asian Pac J Trop Biomed. 2012 Aug; 2(8): 597–601
8. Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria.Park M, Bae J, Lee DS. 2008 Nov;22(11):1446-9. doi: 10.1002/ptr.2473.
9. Attin T, Zirkel C, Pelz K. Antibacterial properties of electron beam sterilized gutta-percha cones. J Endod. 2001; 27:172–4.
10. Subbiah, Subba Rao CV, Balaji RG. Effect of disinfectants and glass bead size on efficacy of glass bead sterlizer. J Conserv Dent. 2005; 8:23–31.
11. Gnau HL, Goodell GG, Imamura GM. Rapid chairside sterilization of endodontic files using 6% Sodium hypochlorite. J Endod. 2009; 35:1253–4.
12. de Souza RE, de Souza EA, Sousa-Neto MD, Pietro RC. In vitro evaluation of different chemical agents for the decontamination of gutta-percha cones. Pesqui Odontol Bras. 2003; 17:75–7.
13. Redmerski R, Bulla JR, Moreno T, Garcia LB, Cardoso CL. Disinfection of gutta-percha cones with chlorhexidine. Braz J Microbiol. 2007; 38:649–55.
14. Siqueira JF, Jr, da Silva CH, Cerqueira M, das D, Lopes HP, de Uzeda M. Effectiveness of four chemical solutions in eliminating Bacillus subtilis spores on gutta percha cones. Endod Dent Traumatol. 1998; 14:124–6.
15. Vinothkumar TS, Rubin MI, Balaji L, Kandaswamy D. In vitro evaluation of five different herbal extract as an antimicrobial endodontic irrigant using real time quantitative polymerase chain reaction. J Conserv Dent. 2013; 16:167–70.
16. Ghonmode WN, Balsaraf OD, Tambe VH, Saujanya KP, Patil AK, Kakde DD. Comparison of the antibacterial efficacy of neem leaf extract, grape seed extract and 3% sodium hypochlorite against E. feacalis: An in vitro study. J Int Oral Health. 2013; 5:61–6
17. Lantz RC, Chen GJ, Sarihan M, Sólyom AM, Jolad SD, Timmermann BN. The effect of extracts from ginger rhizome on inflammatory mediator production. Phytomedicine2007; 14:123-128.