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Effect of Thyme Water Extract on Commonly Found Oral and Root Canal Bacteria (A Comparative Study)

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ABSTRACT

Background and objectives: Bacteria and their products play an essential role in the pathogenesis of pulpo-periapical disease. The purpose of this study was to compare the anti-microbial activity of thyme water extract on selected bacterial isolates and cultivated root canal swabs in comparison with different concentrations of sodium hypochlorite (NaOCl).

Materials and Methods: Clinical isolates of *Staphylococcus aureus*, *Moraxella catarrhalis*, and *Klebsiella pneumoniae* were selected. Microbiological samples from infected root canals were taken from forty five male and female patients in age group of 15-45 years old during the first visit of root canal therapy. Antibacterial assay were done by agar well diffusion method and microdilution assay.

Results: The best antimicrobial activity for thyme extract was noticed at *Moraxella catarrhalis* and failed to show any significant effect on *Klebsiella pneumoniae*. The results revealed that 2% sodium hypochlorite are more effective than thyme water extract, on evaluated bacterial isolates and swabs.

Conclusion: From bacteriological point of view, it could be demonstrated that water extracts of thyme (infusion method), may be useful for root canal irrigation with their antibacterial effect.

KEYWORDS: Thyme, Irrigant, Sodium hypochlorite, Root canal bacteria.

INTRODUCTION:

The goal, with the great majority of teeth required root canal treatment, is either prevention or treatment of apical periodontitis. In other words, the goal is prevention and elimination of a microbial infection in the root canal system.[1] Bacteria and their products play an essential role in the pathogenesis of pulpo-periapical disease.[2] Eradication of microorganisms from an infected root canal system has been demonstrated in numerous studies to be the key to successful endodontic treatment.[3,4]

Although root canal irrigants such as sodium hypochlorite appear to be effective at reducing bacterial cultures, most of the previous studies failed to adequately report these clinically important and potentially patient-relevant outcomes. There is currently insufficient reliable evidence showing the superiority of any one individual irrigant.[5]

The pulpo-dentin complex is normally protected from the oral cavity by the overlying enamel or cementum. Once caries, trauma, or restorative or periodontal procedures breach the integrity of this barrier, the tubules provide diffusion channels from the surface to the pulp. Bacteria can then invade these dentinal tubules, and bacterial products can diffuse across dentin to elicit pulpal reactions.[6] All bacteria within the oral cavity share the same opportunities for invading the root canal space, however only a restricted group of species have been identified in infected root canals.[7] The predominant microbial groups frequently isolated from infected root canal are the aerobic and facultative anaerobic organisms.[8,9] These types of organisms frequently isolated more than obligate anaerobic species in developed periapical lesion, during the standard culture technique.[9,10]

Aromatic plants are promising sources of natural antimicrobial and antioxidant compounds. These properties may be associated with their bioactivities and health effects.[11] Thyme (*Thymus vulgaris L.*) is an aromatic plant known by its antioxidant and antimicrobial activity, both related to the phenolic compounds, particularly *thymol* and *p-cymene*, the most abundant compounds in thyme leaves.[12,13] It is used orally to treat dyspepsia and other gastrointestinal disturbances; coughs due to colds, bronchitis and pertussis; and laryngitis and tonsillitis (as a gargle). Topical applications of thyme extract have been used in the treatment of minor wounds, the common cold, disorders of the oral cavity, and as an antibacterial agent in oral hygiene.[14,15]

The purpose of this study was to evaluate *in vitro*, the antimicrobial activity of thyme water extract, in comparison with sodium hypochlorite

against selected bacterial isolates and against swabs taken clinically from prepared root canals in patients presenting for endodontic treatment.

MATERIALS & METHODS

Determination of Plant Material

Five hundred grams of fresh plant material were purchased from the local botanical market of Sulaimani and were identified by the Herbarium of the College of Agricultural, Sulaimani University.

Preparation of the Plant Extract

Extraction of fresh plant material was performed by hot water extraction, without drying the plant parts according to Goyal, *et al*. (2008).[16] The infusion prepared by boiling 10 g of plant parts (leaf, stem and root) in distilled water with constant stirring for 30min. The solution was allowed to cool to room temperature and then filtered using muslin cloth. The filtrate was centrifuged at 500rpm for 15min. The supernatant was again filtered using Whatman filter paper (No.1) under strict aseptic condition. The filtrate was collected in fresh sterilized glass tubes and stored at 40C until use.

Test Organisms

Clinical isolates of *Staphylococcus aureus, Moraxella catarrhalis*, and *Klebsiella pneumoniae* were used in this study. The microorganisms were provided by microbiology laboratory of the Biology Department, College of Science, Sulaimani University.

Root Canal Sampling

Forty-five male and female patients in age group of 15-45 years attending dental clinic at Conservative Department, College of Dentistry, University of Sulaimani, were taken for this study. Each patient included in study was having at least one intact or necrotic open canal tooth. As a diagnostic measure, for each of the suspected tooth an intra oral periapical X-ray was taken.

Forty-five endodontic samplings from infected root canal were obtained during the first visit of root canal therapy by a sterile file. Sampling was also performed to check and confirm the sterility of the operating field before intracanal sampling procedure, and then transferred to sterile 2ml tubes containing VMGA III transport medium. All samples were processed within 2 hours, and cultivated over a suitable growth media. The culture media used were nutrient agar, Mueller Hinton agar, MacConkey agar, Cetrimide agar, Simmon citrate agar and peptone water (Biotech Laboratories United Kingdom), Thioglycolate broth (Maknur, Canada), Trypticase Soy broth (Becton Dickinson, USA), and the media were prepared according to the manufacturers' specifications. Two sets of plates with different growth media were equally prepared for each patient, one for aerobic incubation, and the other for anaerobic incubation.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MIC) of thyme water extract and NaOCl, against the test bacteria were determined by standard two-fold microdilution methodology as described by Levinson (2004).[17] Thioglycolate medium fluid was prepared according to the manufacturer's instructions. Different concentration of thyme water extract were made from crude extracts (200, 250, 500, 750, 1000, 1500) mg/ml. The dilution procedure was carried out using micropipettes. A sterilized screw-capped tube was used for the dilution, the total volume of contents of each tube after dilution was 4ml.

Antibacterial Activity

An *in vitro* agar well diffusion assay was performed, to test the susceptibility of selected clinical isolates and sub-cultured root canal swabs to thyme water extract and NaOCl.[18] Fifteen milliliters of the molten agar (45 °C) were poured into sterile petri dishes (Ø 90mm).50 µl from each selected bacterial isolate and from the swabs (Cell suspensions containing 10^8 CFU/ml cells), were taken separately and evenly spread onto the surface of the agar plates of Mueller-Hinton agar (Oxoid, UK), using a micropipette. Five plates for each microorganism were inoculated. For each swab two sets of plates with different growth media were inoculated, one for aerobic incubation, and the other for anaerobic incubation. Once the plates had been aseptically dried, 6mm wells were bored using a sterile cork borer. Equal amount (75 µl) of thyme water extract (1500mg/ml), 1%NaOCl and 2%NaOCl were placed into the wells and the plates were incubated at 37°C for 24h.

Statistical Analysis

Growth inhibition zones were calculated. Student *t*-test was applied to determine significant differences between the test and control solutions. Significance was predetermined at $P<0.05$. Statistical analysis was performed with the SPSS statistical software package.

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RESULTS

The descriptive statistics (mean and standard deviation) of the inhibition zones (in millimeter) around the wells for thyme water extract (1500mg/ml), 1%NaOCl and 2%NaOCl against the selected bacterial isolates, and against swabs taken from prepared root canals that were incubated aerobically and anaerobically, are shown in table 1. The best antimicrobial activity of thyme extract and both concentrations of NaOCl were noticed at *Moraxella catarrhalis*. The results revealed that 2% NaOCl had more antimicrobial activity ($p<0.05$) than thyme and 1% NaOCl on the tested microorganisms and the swabs. Also, it was observed that thyme extract was failed to show any significant effect on *Klebsiella pneumonia*. It significantly ($p<0.05$) exhibited a wider inhibition zones than 1% NaOCl against *Staphylococcus aureus* and swabs (anaerobic), but it did so to a lesser extent against *Moraxella catarrhalis* and swabs (aerobic).

Concerning the different concentration of thyme water extract, the limits of minimal inhibitory concentration are between 650-1500mg/ml. The 0.1% NaOCl was determined as a minimum concentration required for inhibiting *Staphylococcus aureus* and *Moraxella catarrhalis*. The MIC of NaOCl for *Klebsiella pneumoniae* and swabs was 1%.

(Table 1) Descriptive statistics (mean and standard deviation) of the inhibition zones (in millimeter) of the thyme water extract, 1% NaOCl and 2%NaOCl against bacterial isolates and swabs.

Test organisms	Thyme(1500 mg/ml) Mean±SD	1% NaOCl Mean±SD	2% NaOCl Mean±SD
Moraxella catarrhalis Neisseria catarrhalis	14 ± 1	12 ±0.71	15.6±1.14
Klebsiella pneumoniae	NE	7 ± 0.71	8.4 ± 0.89
Staphylococcus aureus	9 ± 0.71	7.8 ± 1.3	12.2 ± 0.84
Swab(aerobic)	7.71 ±0.73	7.69 ± 0.7	13.64 ±1.33
Swab(anaerobic)	12.42 ±1.44	8.13 ± 1.06	13.58 ±1.45

NE: the test solution was not effective.

DISCUSSION

The use of antimicrobial agent is still considered the fundamental principles during endodontic treatment. None of the chemomechanical methods of root canal treatment is at present able to eliminate all the bacteria from inside the tooth.[1] Available literature and studies demonstrated advantages and limitations of each irrigant under consideration, and none of them satisfy the requirements of the ideal root canal irrigant completely. The search for an ideal root canal irrigant continues with the development of newer materials and methods.[19]

Therein lies, the overall purpose of this study was a trail to find a natural antimicrobial agent to be used as an adjunct during chemomechanical root canal preparation, to eliminate the uses of chemical irrigants with their cytotoxic effects. It was planned to conduct a microbiological study, with the aim of determining the minimum inhibitory concentration (MIC), and to assess the antibacterial effect of our test solution (thyme water extract), and to compare it with that of sodium hypochlorite (as a control), whose antibacterial action is well-established in the literature. Sodium hypochlorite, at different concentrations, has been used in endodontic due to its tissue dissolution ability and antimicrobial action.[20] The results of the present study supported the antimicrobial efficacy of sodium hypochlorite on the evaluated microbiota.

All clinical isolates were selected for the present study to represent different groups of organisms, including Gram-positive facultative anaerobic (*Staphylococcus aureus*), Gram-negative facultative anaerobic (*Klebsiella pneumoniae*) and Gram-negative aerobes (*Moraxella catarrhalis*). The variation in the antimicrobial action of thyme and NaOCl against different microorganisms could be attributed to the differences in the mode of metabolism of each bacterial isolate, and the differences in the mode of action of each agent, that's some microorganisms, are with respiratory metabolism, some with fermentative metabolism, and some with both. The zones of inhibition may be affected by the diffusibility of the agent through the agar media. Therefore, the size of the inhibition zones does not indicate the absolute antimicrobial effect of a solution.

Our results showed that there were statistically significant differences in the antimicrobial action between the thyme water extract and 2%NaOCl. The data obtained, clearly showed that their antibacterial properties are lower than sodium hypochlorite, for that reason it cannot replace sodium hypochlorite.

This may be due to the fact that our extraction method was suboptimal for obtaining high concentrations of active agents in the extracts. It is therefore, recommended that future investigation will incorporate sequential distillation of mixtures of herbs from different countries, to ensure adequate cover for environmental factors which may affect the level of active ingredients in the extracts. Thymol and its isomer carvacrol, components derived from thyme are classified as monoterpene phenols and have already proven their antimicrobial effect.[21] Other experimental evidence suggests that the *in vitro* activities of thyme preparations are due to the presence of polymethoxy flavones that have antibacterial activity.[22]

In any case, antimicrobial activity is not the only requirement for an endodontic irrigant. Irrigating solutions also possessing the ability to dissolve organic material are desirable in endodontic treatment. When analyzing the antibacterial action of an endodontic irrigating solution, it is necessary to consider its ability to wet the dentin and penetrate dentinal tubules and also its capacity to disrupt the biofilm community.[23] Therefore, there is need for further studies and modifications in thyme water extract (infusion method), to evaluate their biological behaviors on periapical tissues, before it could be used as a root canal irrigant.

CONCLUSION

The present in-vitro study results showed that water extracts of thyme (infusion method), possess antibacterial activities, it might come in useful during chemo-mechanical root canal preparation.

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