

Antimicrobial activity and substantivity of *Uncaria tomentosa* in infected root canal dentin

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Abstract: The aim of this study was to analyze the antimicrobial activity and substantivity of *Uncaria tomentosa* Willd DC (cat's claw, CC) in root dentin contaminated with *Enterococcus faecalis*. Forty-eight human premolars were contaminated with *E. faecalis* (ATCC 29212) and randomly divided into four groups according to the irrigant used during chemomechanical preparation (CMP): CC group: 2% CC gel; CHX group: 2% chlorhexidine digluconate gel (CHX); NaOCl group: 5.25% sodium hypochlorite (NaOCl); and SS group: sterile saline (SS). Microbiological samples were collected before (S1) and after (S2) CMP and after 7 days (S3). Colony-forming units (CFU/mL) at the different sampling times and comparisons among the groups were statistically analyzed by Wilcoxon and Kruskal-Wallis tests ($p < 0.05$). Significant bacterial reduction was achieved in all groups after CMP ($p < 0.05$). Results show no significant difference between S3 and S2 ($p > 0.05$) in the CC and CHX groups. Bacterial load was higher in S3 than in S2 samples ($p < 0.05$) in the NaOCl and SS groups. Our results suggest antibacterial effect of 2% CC gel against *E. faecalis* in infected dentin, in addition to antibacterial substantivity of 2% CC and 2% CHX up to 7 days.

Keywords: Cat's Claw; Sodium Hypochlorite; Chlorhexidine; *Enterococcus faecalis*.

Introduction

Endodontic therapy aims to remove diseased tissue, eliminate bacteria from the root canal system, and prevent recontamination after treatment. These objectives are achieved by biomechanical cleaning and shaping, by obturation of the root canal system, and by placement of coronal restoration.¹ Unfortunately, because of the anatomical complexity of the root canal system, organic and inorganic residues and bacteria cannot be completely removed and often persist.^{2,3,4} For this reason, a wide variety of chemical substances has been tested to minimize bacteria, necrotic tissue and residual debris,^{1,2,3} particularly sodium hypochlorite (NaOCl), the most widely used endodontic irrigant,⁵ and chlorhexidine (CHX),⁴ another auxiliary chemical substance used in endodontic therapy. None of these irrigants has all of the characteristics of an ideal irrigant.

A desirable property of endodontic irrigants concerns their capacity to be absorbed on negatively charged surfaces in the mouth (e.g., enamel, dentin, cement, mucosa, restorative materials), being slowly released from these



retention sites and therefore maintaining prolonged antimicrobial activity.⁵ This process is known as substantivity, and only CHX and tetracycline have had this property so far.⁶ Thus, several studies have examined the activity and potential application of other chemical substances, including natural products.⁷

Uncaria tomentosa (Willd) DC, which is known as cat's claw (CC) because of the small curved thorns in its leaf axil, is one of the most promising medicinal herbs from the Amazon Rainforest. It has anti-inflammatory, antiviral, antibacterial, antioxidant, and immunomodulatory activity.⁸ Moreover, *U. tomentosa* has not shown toxicity at concentrations between 1–10% in the neutral red assay, kenacid blue total protein assay, tetrazolium assay (MTT), and microtox test.⁹ These properties have allowed the Brazilian Unified Public Health System (*Sistema Único de Saúde* – SUS) to include it on its list of phytotherapeutic agents for anti-inflammatory treatment.

However, no studies have examined the antimicrobial potential of CC in an infected dentin model. Therefore, the present *in vitro* study was conducted to analyze the antibacterial activity and substantivity of 2% CC gel in infected root canal dentin. The null hypothesis assumes that there is no significant difference between 2% CC gel, 2% CHX gel, 5.25% NaOCl, and sterile saline solution (SS) in antibacterial activity and substantivity against *Enterococcus faecalis*.

Methodology

Selection and standardization of specimens

The protocol (155/2014) for sample collection was approved by the Human Research Ethics Committee of the Piracicaba Dental School. Forty-eight human single-rooted premolars were used in the study. The crowns of all teeth were removed with a water-cooled diamond saw (Diamond Wafering Blade, Series 15LC, Buehler, USA) and their roots were standardized to a length of 15 millimeters. The root canals and the apical foramen were enlarged with K-files (Dentsply-Maillefer, Ballaigues, Switzerland) up to size 25, under irrigation with distilled water. The smear layer formed in the canal walls during instrumentation was removed

using an ultrasonic bath with 17% EDTA for 10 min followed by 5.25% NaOCl for 10 min, 5% sodium thiosulfate for 10 min and, finally, distilled water for 1 hour. The specimens, in groups of five, were placed into glass tubes containing 5 mL of SS and autoclaved for complete specimen sterilization.

Specimen contamination and experimental groups

E. faecalis (ATCC 29212) was subcultured onto BHI-blood agar plates (BHI, Lab M, Bury, UK) to obtain fresh colonies, which were adjusted spectrophotometrically at 800 nm in tubes containing 5 mL of SS to match the turbidity of 1.5×10^8 CFU mL⁻¹ and used as the test microorganism. The root canals were individually contaminated for 21 days in tubes containing 5 mL of *E. faecalis* suspension.

The specimens were randomly divided into four groups (n = 12) using the Research Randomizer Software (available at <http://www.randomizer.org>) to define the irrigant to be used during chemomechanical preparation (CMP): CC group: 2% CC gel; CHX group: 2% CHX gel (Endogel, Itapetininga, Brazil); NaOCl group: 5.25% NaOCl; and SS group: SS. A freeze-dried extract of CC (Fleming Manipulações, Ponta Grossa, Brazil) was used to prepare a hydroalcoholic solution of CC mixed with 1% hydroxyethylcellulose (Natrosol) as a gel base.

CMP and microbiological sampling

After contamination, initial samples (S1) were collected using sterile paper points retained in position during 60 seconds. Ten-fold serial dilutions were prepared up to 10⁻⁴ in tubes containing BHI broth (Lab M, Bury, UK) and 50 µL was plated onto BHI-blood agar Petri plates and incubated (37°C/48 h). Thereafter, colony-forming units (CFU) were visually quantified for each plate.

After S1, all specimens were instrumented using the MTwo rotary system (VDW, Munich, Germany) up to # 40.04 file. Prior to the use of a new instrument, the canal was irrigated with 1 mL of the specific substance (CC, CHX, NaOCl, or SS). After the use of each instrument, 1 mL of SS was used for irrigation in all groups. After CMP, all specimens received irrigation with 17% EDTA for 3 min and a final flush with 5 mL of SS. Root canals were lightly dried and

the second samples (S2) were collected and processed as described previously.

Afterwards, the specimens were kept filled with SS and transferred onto plates with 24 flat-bottomed wells (Corning Cell Culture, Corning, USA) and the root canal orifices were sealed using previously disinfected wax. The wells were filled with BHI broth to the surface of the specimens and incubated (37°C/7 days) before sampling and processed as described previously.

Before that, the second and third samples (S2 and S3) were serially diluted and plated. The paper points corresponding to CHX and NaOCl groups were individually soaked in their specific neutralizer [5% Tween 80 and 0.07% (w/v) lecithin, for the CHX group; 5% sodium thiosulfate, for the NaOCl group] for 1 min to avoid any residual antimicrobial activity during culture procedures.

Statistical analysis

The data collected from CFU concentrations were statistically analyzed using the BioEstat 5.3 software (Belém, Pará, Brazil). The Shapiro-Wilk test showed that the data distribution deviated from normality. The Friedman test was performed to compare the amount of bacteria at each sampling time (S1, S2, and S3). The Wilcoxon test was used when significant differences were found between different sampling times. Comparison of the root canal treatment groups (CC, CHX, NaOCl, and SS) was performed by using the Kruskal-Wallis test. The significance level was set at 5% ($p < 0.05$).

Results

Table provides an overview of the amount of culturable bacteria (CFU/mL). The percentage values of bacterial reduction found in all groups tested at different sampling times are shown in Figure.

Culturable bacteria were recovered from 100% (48/48) of the baseline root canal samples (S1). Significant median percentage values of bacterial reduction were obtained in all groups after CMP compared with the baseline samples ($p < 0.05$). Negative bacterial culture was detected in nine S2 samples (75.00%) of the CC group, in 10 samples (83.33%) of the CHX and NaOCl groups, and in two samples (16.66%) of the SS group.

Regarding antibacterial substantivity, no statistically significant difference was observed between the median values of bacterial reduction found in S3 compared with S2 samples in the CC and CHX groups ($p > 0.05$). The NaOCl and SS groups showed a higher bacterial load in S3 compared with S2 samples ($p < 0.05$).

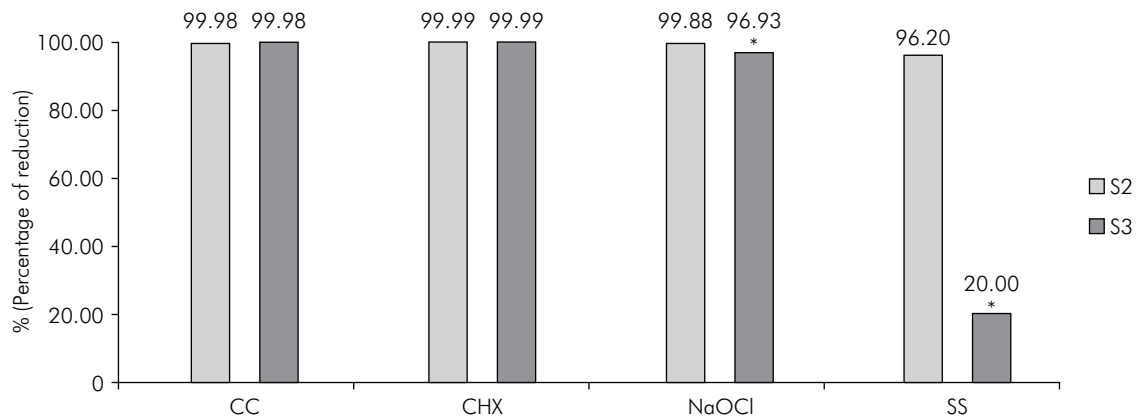
Discussion

The compounds found in CC include oxindole alkaloids, triterpenes, vegetable steroids, phenolic compounds, glycosides, tannin, and flavonoids. These compounds may be related to the antimicrobial activity of CC.^{10,11} Isopteropodine-HCl, a pentacyclic oxindole alkaloid isolated from the bark of the plant, has been shown to be the most potent of the tested compounds, with antibacterial activity against Gram-positive bacteria.¹²

In this study, CC was tested against *E. faecalis*, which is a facultative anaerobic Gram-positive coccus present in 24%–74% of asymptomatic and persistent endodontic infections.¹³ Some of the possible reasons contributing to the resistance of *E. faecalis* to endodontic therapy are its ability to survive long periods of nutritional deprivation and its ability to contaminate dentinal tubules and bind to dentin and collagen.^{13,14}

Specific neutralizers were used to avoid any residual antimicrobial activity of CHX and NaOCl during culture procedures. Since there are no reports about CC inhibition, pilot assays were performed to assess the residual antimicrobial activity of CC in paper points. No inhibitory effect was observed after the use of 17% EDTA.

The data obtained in the present study revealed that regardless of the irrigant used during CMP, there was a significant reduction in the bacterial load. Although this bacterial load reduction was substantial (above 90%) for all groups, culturable bacteria were still detected in 90% of the root canals irrigated with SS (control group), highlighting the importance of the antimicrobial activity of chemical substances. When CC, CHX, or NaOCl were used as irrigants, negative bacterial culture was detected in 29 out of 36 samples (80.56%). The limited ability of root canal treatment to eliminate bacteria has been demonstrated by previous studies in which culturable bacteria were recovered in 20%–60% of the infected teeth after CMP.^{15,16,17,18,19}



CFU: Colony-forming units; CC: cat's claw; CHX: chlorhexidine; NaOCl: sodium hypochlorite; SS: sterile saline.

Figure. Distribution of percentage values found for the reduction of culturable bacteria after CMP (S2) and after 7 days (S3), as well as statistical significance (*).

Table. Median values of bacterial reduction (CFU/mL) obtained from CC, CHX, NaOCl, and SS.

	S1	S2	S3
CC	1.15×10^5 (2.4×10^4 – 1.9×10^5)	1.8×10 (0– 2.1×10)	1.9×10 (0– 2.3×10)
CHX	1.30×10^5 (4.8×10^4 – 1.8×10^5)	1.6×10 (0– 2.0×10)	1.8×10 (0– 2.2×10)
NaOCl	1.11×10^5 (9.5×10^3 – 2.1×10^5)	1.3×10 (0– 2.3×10)	3.4×10^3 (0– 3.7×10^4)
SS	1.00×10^5 (2.1×10^4 – 1.6×10^5)	3.8×10^3 (0– 4.2×10^4)	8.0×10^4 (2.7×10^4 – 3.9×10^5)

CFU: Colony-forming units; CC: cat's claw; CHX: chlorhexidine; NaOCl: sodium hypochlorite; SS: sterile saline; S1: before chemomechanical preparation (CMP); S2: after CMP; S3: after 7 days.

Regarding the substantivity of chemical substances used in this study, CC and CHX prevented bacterial recontamination up to 7 days. Root canals irrigated with NaOCl were recontaminated, showing that the antimicrobial activity of NaOCl could have a limited use in CMP. The data on the substantivity of CHX are consistent with those of previous studies that reported antimicrobial activity for 7 days,²⁰ 21 days,²¹ 4 weeks,⁶ and up to 12 weeks.²² Further studies are necessary to understand how CC chemical molecules interact with dentin to maintain its antimicrobial activity.

Further studies are also necessary to assess the CC capability to neutralize bacterial endotoxins, antimicrobial activity against multi-species biofilms, and possible chemical interactions with other substances commonly used in endodontic therapy.

Conclusion

In conclusion, our results confirm the antibacterial effect of 2% CC gel against *E. faecalis* in infected root

canal dentin. This study also showed the antibacterial substantivity of 2% CC and 2% CHX.

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