

## ***In vitro* antimicrobial activity of phytotherapeutic *Uncaria tomentosa* against endodontic pathogens**

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**Abstract:** The aim of this study was to evaluate the antimicrobial activity of *Uncaria tomentosa* (Willd.) DC (cat's claw) against *Enterococcus faecalis*, *Staphylococcus aureus*, and *Candida albicans*. Suspensions with  $10^8$  cells/ml of each microorganism were plated in triplicate on Mueller-Hinton agar. Wells in the agar were made and filled with 2% chlorhexidine (CHX) gel, 2% cat's claw (CC) gel, 2% CHX+CC, and 1% hydroxyethylcellulose (NAT) gel. Inhibition halos were measured after 24 h at 37°C and differences were analyzed using one-way ANOVA. The mean diameter of the microbial growth inhibition zones of 2% CHX+CC against the tested microbial strains ranged from 21.7 to 33.5 mm. This was the most effective substance against *E. faecalis* and *C. albicans*, followed by CHX and CC. Against *S. aureus*, CHX+CC, CHX, and CC showed similar antimicrobial activity ( $P > 0.05$ ). The results indicate that all the investigated compounds had antimicrobial activity against microorganisms frequently found in infected root-filled teeth. (J Oral Sci 52, 473-476, 2010)

**Keywords:** *Uncaria tomentosa*; auxiliary substance; *Enterococcus faecalis*; *Staphylococcus aureus*; *Candida albicans*.

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### **Introduction**

Endodontic therapy aims to remove diseased tissue, eliminate bacteria present in the canals and dentinal tubules, and prevent recontamination after treatment. These objectives are achieved by biomechanical cleaning and shaping of the root canal system, by sealing root canals with a 3-dimensional obturation, and by placing a coronal seal (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 (2-4). For this reason, a wide variety of irrigants and intracanal medicaments has been developed to minimize residual debris, necrotic tissue, and bacteria, and to remove the smear layer formed by the mechanical preparation of the dentin (4-6).

Chlorhexidine gluconate (CHX) is a broad-spectrum antimicrobial agent that is reported to be an effective medication in endodontic treatment (7-9). The antimicrobial effectiveness of 2% CHX (solution or gel) was thoroughly demonstrated *in vitro* (10). In addition, CHX was shown to have antimicrobial activity against organisms commonly isolated from the root canal system (11).

An increasing number of studies have examined the activity and possible applications of new and natural substances for root canal disinfection (12). Phytotherapy is a viable alternative to satisfy this objective. Currently, the most promising medicinal Amazonian herb is *Uncaria tomentosa* (Willd.) DC, which is known as cat's claw (CC) because of the small curved spines on the stem at the leaf juncture. It has anti-inflammatory, antiviral, antibacterial, antioxidant, and immunomodulating activity (13). Studies of the chemical and pharmacological

properties of this medicinal plant have allowed researchers to develop indications for its use. Its toxicity is low when used correctly, which is an important advantage of medicinal plant treatments (14). No studies have examined the antimicrobial potential of CC in endodontic therapy. Thus, the aim of this *in vitro* study was to evaluate the susceptibility to *U. tomentosa* of microorganisms frequently found in infected root-filled teeth.

## Materials and Methods

Four auxiliary substances were evaluated: 2% CHX gel, 2% CC gel, 2% CHX + 2% CC gel (1:1/v:v), and 1% hydroxyethylcellulose (NAT) gel (negative control). The experimental substances were prepared using 2% aqueous solutions of CHX and CC (Fleming Manipulações, Ponta Grossa, PR, Brazil) and 1% NAT as gel base (Fleming Manipulações), commercially known as Natrosol. A freeze-dried extract of CC (Fleming Manipulações) was used to prepare a 2% aqueous solution, which was mixed with NAT to obtain a 2% CC gel.

The microorganisms used in this study were *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 95106), and *Candida albicans* (ATCC 10231). All microorganism strains were obtained from the stock of the Laboratory of Microbiology, School of Dentistry, University Estadual de Ponta Grossa, PR, Brazil.

The agar diffusion method was used to measure the antimicrobial activity of the compounds. All microorganisms were subcultured in brain-heart infusion (BHI, Lab M, Bury, UK) agar to obtain fresh strains that were adjusted spectrophotometrically at 800 nm in tubes containing 5 ml of sterile saline to match the turbidity of  $1.5 \times 10^8$  CFU ml<sup>-1</sup> (equivalent to 0.5 McFarland turbidity standard). Then, 100 µl of each microbial suspension was plated with a swab on Mueller-Hinton agar (Oxoid, Unipath Ltd, Basingstoke, UK) and incubated at 37°C for 10 min for drying. Sterile glass tubes that were open at one end and had an outside diameter of 4 mm and a wall thickness of 0.8 mm were used to make 4 wells on the media. Each

of the wells was filled with a test substance (CHX, CC, or CHX+CC) or the negative control (NAT).

The media were incubated at 37°C for 24 h to allow the microorganisms to grow and the reagents to diffuse throughout the culture medium, after which the inhibition zones around wells containing the investigated substances were measured with a digital caliper. The inhibition zone was defined as the shortest distance (mm) between the outer margin of the well containing the auxiliary substance and the initial point of microbial growth. Three replicates were made for each microorganism, and the values were analyzed by one-way ANOVA. The significance level was set at 5%.

## Results

The results of *in vitro* evaluation of the antimicrobial activity of the auxiliary substances are presented in the Table 1 and Fig. 1. The mean diameter of the microbial growth inhibition zones of 2% CHX+CC against the tested microbial strains ranged from 21.7 to 33.5 mm. It was the most effective substance against *E. faecalis* and *C. albicans*, followed by CHX and CC. CHX+CC, CHX, and CC had similar antimicrobial activity against *S. aureus* ( $P > 0.05$ ). As expected, NAT had no inhibitory effect.

## Discussion

CC is the best known Peruvian medicinal plant and the most frequently studied (13). It contains oxindole alkaloids, triterpenes, vegetal steroids, phenolic compounds, glycosides, tannin, and flavonoids. These compounds may be related to its antimicrobial activity (15). Isopteropodine-HCl, a pentacyclic oxindole alkaloid isolated from the bark of the plant, was shown to be the most potent of the tested compounds and has antibacterial activity against Gram-positive bacteria (16).

In this study, we used the agar diffusion test, which is the most widely used *in vitro* method for the evaluation of antimicrobial activity. This method allows direct comparisons between irrigants and intracanal medicaments (17). Diffusion *in vitro* allows us to estimate potential

Table 1 Mean inhibition zone values (± standard deviation) for the evaluated substances

Auxiliary substance	Mean inhibition zone (mm)		
	<i>E. faecalis</i>	<i>S. aureus</i>	<i>C. albicans</i>
CHX	20.5 ± 0.2 B	24.4 ± 0.3 E,F	31.7 ± 0.6 I
CC	18.9 ± 0.1 C	25.4 ± 0.4 E	26.5 ± 0.2 J
CHX+CC	21.7 ± 0.7 A	25.1 ± 0.3 F	33.5 ± 0.5 H
NAT	0.0 ± 0.0 D	0.0 ± 0.0 G	0.0 ± 0.0 K

Among the treatment groups of each microbial strain, means followed by the same letters are not significantly different (ANOVA,  $P > 0.05$ ).

Abbreviations, CHX: 2% chlorhexidine gel; CC: 2% cat's claw gel; NAT: 1% hydroxyethylcellulose.

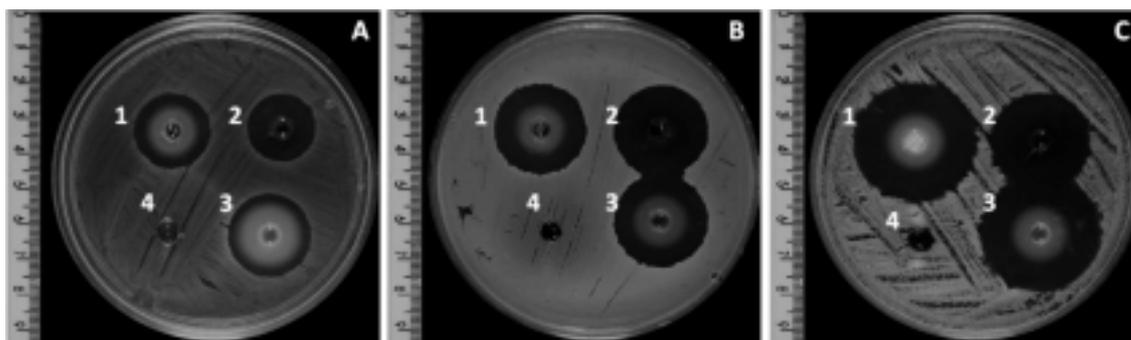


Fig. 1 Inhibition zones against *Enterococcus faecalis* (A), *Staphylococcus aureus* (B), and *Candida albicans* (C) promoted by CHX (1), CC (2), CHX+CC (3), and NAT (4).

Abbreviations, CHX: 2% chlorhexidine gel; CC: 2% cat's claw gel; NAT: 1% hydroxyethylcellulose.

diffusion into the root canal, which is a desirable property for intracanal irrigants and medicaments (18). The choice of *E. faecalis*, *S. aureus*, and *C. albicans* for this study is justified by considerable previous research, as these are the most resistant species in the oral cavity and are frequently associated with failure of root canal treatment (19,20).

CHX was selected as a positive control in this study because it has been thoroughly investigated in endodontic microbiology, due to its potent antimicrobial capacity and high biocompatibility (21). In the experimental groups, the gel form was used because gels can serve as root canal lubricants, which promote better removal of organic debris (22), and because gels can be used as an intracanal medicament. In the present study, hydroxyethylcellulose was used as the gel base for the auxiliary substances, as it was shown to be an inert substance without antimicrobial activity (22), a finding that was confirmed by our results.

The choice of a 2% concentration for CC gel was based on the findings from a minimum inhibitory concentration (MIC) pilot assay in which CC concentrations of 0.5%, 1%, 2%, and 5% were tested: the antimicrobial results for the 2% and 5% concentrations were similar. Ccahuana-Vasquez et al. (23) used MIC assays to evaluate CC antimicrobial activity in concentrations ranging from 0.25% to 5%. The authors related antimicrobial activity of 2% CC against *S. aureus* strains, which agrees with the results of this study. In the same study, the authors observed no CC antimicrobial activity against *C. albicans*, which differs from our results for that microorganism. A possibly reason for this difference is that we used a gel obtained from a freeze-dried extract, in which active compounds of CC may have been more stable, as compared with the micropulverized extract used by Ccahuana-Vasquez et al. Variations in plant material and extraction procedures

might affect the concentrations of active compounds (24), which could be reflected in antimicrobial activity.

Studies have suggested that CHX gel is an effective intracanal medication due to its broad antimicrobial spectrum (17,22,25), which is in agreement with the findings of the present study. Our results showed that CHX+CC was the most effective substance against *E. faecalis* and *C. albicans*, producing the largest mean inhibition zones. *In vitro* studies have demonstrated that some substances, such as Ca(OH)<sub>2</sub>, urea, and sodium lauryl sulphate, reduce the antimicrobial activity of CHX (26). Our results show that CC is a natural substance that does not appear to affect the antimicrobial activity of CHX. Indeed, the use of CC may have a synergistic effect that increases the substantivity of CHX.

Further multidisciplinary studies are necessary to identify precisely which chemical compounds of CC have the antimicrobial potential observed in this study. Possible interactions between CC and other auxiliary substances commonly use in endodontic therapy must be evaluated to determine possible synergistic or adverse reactions. Moreover, additional clinical trials are necessary to confirm the results of *in vitro* studies.

Our results suggest that 2% CC gel inhibits microorganisms frequently found in infected root-filled teeth and that this effect may be increased when CC is combined with CHX.

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