

Antimicrobial activity of *Uncaria tomentosa* against oral human pathogens

Atividade antimicrobiana da *Uncaria tomentosa* sobre patógenos da cavidade bucal humana

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Abstract: *Uncaria tomentosa* is considered a medicinal plant used over centuries by the peruvian population as an alternative treatment for several diseases. Many microorganisms usually inhabit the human oral cavity and under certain conditions can become etiologic agents of diseases. The aim of the present study was to evaluate the antimicrobial activity of different concentrations of *Uncaria tomentosa* on different strains of microorganisms isolated from the human oral cavity. Micropulverized *Uncaria tomentosa* was tested *in vitro* to determine the minimum inhibitory concentration (MIC) on selected microbial strains. The tested strains were oral clinical isolates of *Streptococcus mutans*, *Staphylococcus* spp., *Candida albicans*, Enterobacteriaceae and *Pseudomonas aeruginosa*. The tested concentrations of *Uncaria tomentosa* ranged from 0.25–5% in Müeller-Hinton agar. Three percent *Uncaria tomentosa* inhibited 8% of Enterobacteriaceae isolates, 52% of *S. mutans* and 96% of *Staphylococcus* spp. The tested concentrations did not present inhibitory effect on *P. aeruginosa* and *C. albicans*. It could be concluded that micropulverized *Uncaria tomentosa* presented antimicrobial activity on Enterobacteriaceae, *S. mutans* and *Staphylococcus* spp. isolates.

Descriptors: Cat's claw; Contact inhibition; Bacteria; *Candida albicans*.

Resumo: *Uncaria tomentosa* é uma planta medicinal usada por vários séculos pela população peruana como alternativa de tratamento para diversas doenças. Muitos microrganismos que usualmente não habitam a cavidade bucal humana podem se tornar agentes etiológicos de doenças sob certas condições. O objetivo deste estudo foi avaliar a atividade antimicrobiana de diferentes concentrações de *Uncaria tomentosa* sobre diferentes cepas de microrganismos isolados de cavidades bucais humanas. *Uncaria tomentosa* micropulverizada foi testada *in vitro* para determinar a concentração inibitória mínima (CIM) em isolados microbianos selecionados. Cepas de *Streptococcus mutans*, *Staphylococcus* spp., *Candida albicans*, Enterobacteriaceae e *Pseudomonas aeruginosa* avaliadas foram isoladas de cavidades bucais humanas. Foram preparadas as concentrações de *Uncaria tomentosa* entre 0.25 e 5% em ágar Müeller-Hinton. *Uncaria tomentosa* a 3% inibiu 8% de Enterobacteriaceae, 52% de *S. mutans* e 96% de *Staphylococcus* spp. As concentrações testadas não apresentaram efeito inibitório sobre *P. aeruginosa* e *C. albicans*. Concluiu-se que *Uncaria tomentosa* micropulverizada apresenta atividade antimicrobiana sobre cepas de Enterobacteriaceae, *S. mutans* e *Staphylococcus* spp.

Descritores: Unha-de-gato; Inibição de contato; Bactérias; *Candida albicans*.

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Introduction

Dental caries is defined as an imbalance in the processes of demineralization and remineralization of the hard tissues of the tooth. Loss of mineral from the hard tissues occurs due to a higher grade of demineralization. Dental caries is a multifactorial pathology which involves a susceptible host, a cariogenic microbiota and a cariogenic diet. These factors must occur simultaneously during a certain period of time for the occurrence of dental caries.¹⁶ *Streptococcus mutans* is considered the main etiological agent of caries of smooth surfaces in humans and in animal models. Previous studies showed that *mutans* streptococci are involved in the initial stages of dental demineralization.^{3,16}

Other species of microorganisms that are present in the oral cavity such as *Candida albicans* and *Staphylococcus aureus* may also cause pathologies, particularly under specific conditions. Microorganisms of the Enterobacteriaceae family and of the genus *Pseudomonas* have been extensively studied due to their pathogenic potential. Also, these bacteria are correlated to severe periodontal diseases.^{22,23}

Treatment of caries and periodontitis has been performed by clinical procedures. The limitations of the mechanical control of dental biofilm originated several studies on the activity of chemical agents. These products can act interfering in the bacterial adhesion to the dental surface, reducing bacterial proliferation or removing the pre-existing biofilm.¹⁶

Nowadays, an increase in the number of investigations on new and natural substances in order to evaluate their activity and possible application to control the dental biofilm is observed.²⁷

Uncaria tomentosa is a medicinal plant used over the centuries by the indigenous civilization of the peruvian rainforest, as an alternative treatment for different diseases. Studies performed in the last decades proved its antiinflammatory,^{5,8,17,25} antineoplastic,^{19,20} anticonceptive,¹² immunostimulant¹⁸ and antioxidant^{4,11,13} properties.

Fitotherapy is a viable alternative for all health professionals in the prevention and treatment of several pathologies. The use of medicinal plants has low cost and has been used for several generations.² Scientific studies on the chemical and pharmacological

properties of medicinal plants allow scientists to indicate their proper use. Low toxicity, when correctly employed, is considered one of the main advantages of the treatment with medicinal plants.^{8,14,26} Thus, the aim of the present study was to analyze the effects of *Uncaria tomentosa* on isolates from the human oral cavity.

Material and Methods

Micropulverized *Uncaria tomentosa* (Willd.) DC. originated from Peru was commercially obtained and used in this study (100% purity, Farma & Fórmula, Taubaté, SP, Brazil, batch 009).

One hundred and six strains from the human oral cavity, isolated and phenotypically identified in previous studies^{6,7,15}, were included in the experiments. These isolates were maintained in the Collection of Cultures of the University of Taubaté - CCUT (Table 1).

Streptococcus mutans strains were previously inoculated on plates of Mitis Salivarius agar (Difco, Detroit, Michigan, USA); *Staphylococcus* spp., on Baird-Parker agar (Difco, Detroit, Michigan, USA), *Candida albicans*, on Sabouraud dextrose agar

Table 1 - Isolates included in the study for the determination of the minimum inhibitory concentration (MIC) of *Uncaria tomentosa*.

Genus	Species	Number of strains
<i>Streptococcus</i>	<i>S. mutans</i>	25
<i>Staphylococcus</i>	<i>S. aureus</i>	22
	<i>S. intermedius</i>	3
<i>Candida</i>	<i>C. albicans</i>	25
<i>Klebsiella</i>	<i>K. pneumoniae</i>	5
	<i>K. oxytoca</i>	2
	<i>K. terrigena</i>	1
<i>Enterobacter</i>	<i>E. cloacae</i>	5
	<i>E. sakazakii</i>	2
	<i>E. asburiae</i>	1
	<i>E. amnigenus</i>	1
<i>Escherichia</i>	<i>E. coli</i>	2
<i>Citrobacter</i>	<i>C. freundii</i>	1
	<i>C. amalonaticus</i>	1
<i>Serratia</i>	<i>S. liquefaciens</i>	1
	<i>S. odorifera</i>	1
<i>Pantoea</i>	<i>Pantoea</i> spp.	2
<i>Pseudomonas</i>	<i>P. aeruginosa</i>	6
Total		106

(Difco, Detroit, Michigan, USA) and Enterobacteriaceae/*Pseudomonas aeruginosa*, on MacConkey agar (Difco, Detroit, Michigan, USA). The plates were incubated at 37°C for 24 hours (and 5% CO₂, for *Streptococcus mutans*). After the period of incubation, standardized suspensions were obtained in 10 ml of sterile saline solution (0.85% NaCl) and adjusted to a turbidity of McFarland No. 1 barium sulfate standard.

Minimum inhibitory concentration (MIC) of *Uncaria tomentosa* was obtained according to the method of dilution in Müller-Hinton agar (Difco, Detroit, Michigan, USA). A series of plates containing Müller-Hinton agar and *Uncaria tomentosa* at 0.25%, 0.5%, 1%, 2%, 3%, 4% and 5% were prepared. Plates of Müller-Hinton agar without micropulverized *Uncaria tomentosa* were included as positive control; 2 µg/mL ciprofloxacin and 1 µg/mL amphotericin B were used as negative control for bacterial and fungal strains, respectively.

The suspensions of the different microbial strains were inoculated with the aid of a Steers replicator and were incubated at 37°C for 24 hours (and 5% CO₂ for *S. mutans*). Readings were performed considering the presence or absence of growth according to Oplustil⁹ (2000). The MIC of micropulverized *Uncaria tomentosa* that was able to inhibit the growth of the isolates was recorded. All the experiments were performed in duplicate.

Results

The results obtained on the inhibitory activity of the different concentrations of *Uncaria tomentosa* against the tested strains can be observed in Table 2.

Minimal inhibitory concentration (MIC) of *Uncaria tomentosa* was 3% (n = 13) for 52% of the tested *S. mutans* isolates. Five percent *Uncaria tomentosa* was the MIC for 60% of the isolates (n = 15).

Twenty-two strains of *S. aureus* and 3 strains of *S. intermedius* were evaluated (Table 1). Growth of all strains was observed at the concentrations of 0.25%, 0.5% and 1% *Uncaria tomentosa*. Two percent *Uncaria tomentosa* was the MIC for 88% (n = 22) of the *Staphylococcus* isolates, including 3 strains of *S. intermedius*. Twenty-four (96%) of the *Staphylococcus* isolates were inhibited by 3% *Uncaria tomentosa*. *S. aureus* CCUT 101025 was resistant to all the tested concentrations.

Among the Enterobacteriaceae isolates only one *Klebsiella pneumoniae* (ATCC 18883) and *Citrobacter freundii* (CCUT 22001) were inhibited by 2% and 3% *Uncaria tomentosa*, respectively.

The concentrations of *Uncaria tomentosa* tested in the present study did not inhibit the growth of the *C. albicans* and *P. aeruginosa* isolates included in this study.

Discussion

Antimicrobial properties of several plants have been investigated as alternatives with low toxicity for the prevention and treatment of infectious diseases. *Uncaria tomentosa* presents components like oxindole alkaloids, triterpens, vegetal steroids, phenolic compound, glycosids, tanin and flavonoids. These compounds may be related to its microbial activity.^{1,8,10}

In the present study, the MIC value of 3% *Uncaria tomentosa* was registered for 52% of the *S. mutans*

Table 2 - Susceptibility of the tested microorganisms to *Uncaria tomentosa*.

Microorganisms	Number of tested isolates	Number of inhibited isolates	Number of strains with MIC (%)						
			0.25%	0.5%	1%	2%	3%	4%	5%
<i>Streptococcus mutans</i>	25	15	0	0	0	0	13	1	1
<i>Staphylococcus</i> spp.	25	24	0	0	0	22	2	0	0
<i>Candida albicans</i>	25	0	0	0	0	0	0	0	0
Enterobacteriaceae	25	2	0	0	0	1	1	0	0
<i>Pseudomonas aeruginosa</i>	6	0	0	0	0	0	0	0	0

MIC - minimum inhibitory concentration.

strains. Concentrations of 4% and 5% of *Uncaria tomentosa* inhibited 56% and 60% of these microorganisms, respectively. The literature does not register previous studies on the use of this plant against *S. mutans* and other oral microorganisms. Differences in the susceptibility of the isolates to *Uncaria tomentosa* may be probably related to possible genotypic variations that might be further investigated.

The concentrations of 2 to 3% of *Uncaria tomentosa* inhibited from 88 to 96% of the strains of *Staphylococcus* spp. This was the major inhibition index registered among the tested microorganisms. Out of the 24 *Staphylococcus* isolates inhibited, twenty-one were *S. aureus*. This microorganism is related to nosocomial infections, and shows resistance to several antimicrobials.⁵ These results suggest that the possibility of the use of *Uncaria tomentosa* against this microorganism is very promising and should be better studied. Moreover, Valerio, Gonzales²⁴ (2005) cited that animal toxicological studies indicated low potential for acute and sub-acute oral toxicity of *Uncaria tomentosa*.

No activity of *Uncaria tomentosa* on *C. albicans* growth was observed in this study. This result differs from that presented by Silva *et al.*²¹ (1998). These authors registered 66% of inhibition of *C. albicans* isolates. This difference may be related to the concentration and type of *Uncaria tomentosa* employed in the experiments. In this study we used micropulverized *Uncaria tomentosa* up to 5% (50 mg/

ml). Silva *et al.*²¹ (1998) used a freeze-dried extract at a concentration of 500 mg/ml.

Three percent *Uncaria tomentosa* was effective to inhibit the growth of 8% of Enterobacteriaceae (2/25) isolates, being one strain of *C. freundii* (CCUT 22001) and one of *K. pneumoniae* (ATCC 18833). Silva *et al.*²¹ (1998) evaluated a freeze-dried extract of *Uncaria tomentosa* on *E. coli* isolates and observed growth inhibition with disks containing 8.7 mg. In this study, 2 strains of *E. coli* were tested (CCUT 71003 and ATCC 25922) and no inhibition was observed.

No growth inhibition of *P. aeruginosa* was observed even at the concentration of 5% of *Uncaria tomentosa*. *P. aeruginosa* is an important pathogen with high resistance to different compounds and also several antimicrobials.¹⁵

More detailed studies must be developed on the identification of chemical compounds of *Uncaria tomentosa* and the correlation with the antimicrobial activity. Data on the antimicrobial activity on pathogens are important for the future application of this plant in medicine and dentistry.

Conclusions

It could be concluded that micropulverized *Uncaria tomentosa* presented antimicrobial activity on Enterobacteriaceae, *S. mutans* and *Staphylococcus* spp. isolates, however it did not present inhibitory effect on *P. aeruginosa* and *C. albicans*.

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