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INFORME BREVE

Antimicrobial activity of yerba mate (*Ilex paraguariensis* St. Hil.) against food pathogens

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Abstract
Yerba mate (*Ilex paraguariensis* St. Hil.) has been studied for its important biological activities mainly attributed to phenolic compounds. This study evaluated the antimicrobial activity of methanolic and ethanolic extracts of yerba mate against food pathogens, such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* Enteritidis and *Escherichia coli* through minimum inhibitory (MIC) and bactericidal (MBC) concentrations, in addition to the determination of chemical composition by gas chromatography with mass spectrometry (GC-MS) and phenolic content. The most effective extract had its activity evaluated under different pH conditions by growth curve analysis. All microorganisms except *E. coli* were inhibited. The ethanolic extract showed the lowest MIC/MBC (0.78/0.78 mg/ml), the highest phenolic content (193.9 g.GAE/kg) and the presence of chlorogenic acid derivatives, especially 3-O-caffeoylquinic and caffeic acid. This extract was able to inhibit microbial growth at pH 7 and 8.

PALABRAS CLAVE
Yerba mate;
Actividad antimicrobiana;
Staphylococcus aureus;
Listeria monocytogenes;
Salmonella Enteritidis

Actividad antimicrobiana de la yerba mate (*Ilex paraguariensis* St. Hil.) contra patógenos alimentarios

Resumen
La actividad biológica de la yerba mate (*Ilex paraguariensis* St. Hil.) ya ha sido descrita. Dicha actividad generalmente se ha asociado a la presencia de compuestos fenólicos. Este estudio evaluó la actividad antimicrobiana de los extractos etanólicos y metanólicos de la yerba mate contra patógenos alimentarios como *Staphylococcus aureus*, *Listeria*

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monocytogenes, Salmonella Enteritidis y *Escherichia coli* mediante la determinación de la concentración inhibitoria (CIM) y bactericida mínima (CBM). También se efectuó el análisis de la composición química por cromatografía gaseosa-espectrometría de masas (CG-EM) y se determinó el contenido de compuestos fenólicos. El extracto con mayor capacidad inhibitoria se evaluó en diferentes condiciones de pH, por análisis de curvas de crecimiento. Todos los microorganismos fueron inhibidos, excepto *E. coli*. El extracto etanólico mostró la menor CIM/CBM (0,78/0,78 mg/ml), el más alto contenido de fenólicos totales (193,9 g.EAG/kg) y la presencia de derivados clorogénicos, principalmente ácido 3-O-cafeoilquínico y cafeico. Este extracto fue capaz de inhibir el crecimiento microbiano a pH 7 y 8.

In recent decades, research has shown the potential exploitation of plant products as a bioactive compound source for industrial interest. Leaves, stems and flowers may present biological activities[1]. In addition, the study of products used in infusions such as teas and beverages has gained increasing prominence[14]. Among these products, yerba mate stands out, being a product widely used by South American populations both as a source of caffeine in place of or in addition to tea and coffee; also as a therapeutic agent due to its known pharmacological properties such as antioxidant, anti-inflammatory, antitumor, and weight reducing activities[2].

In the last years, different investigations have revealed the antimicrobial potential of yerba mate, whose spectrum of activity includes gram-positive and gram-negative bacteria and fungi[4,5]. These bioactivities are strictly related to the presence of different classes of compounds, mainly phenolics, whose main representatives in yerba mate are gallic, syringic, caffeic, ferulic and ρ-coumaric acids[11]. However, there are few studies about the influence of pH conditions on the activity of these compounds in crude extracts; these data are extremely important, since this variable is vital for antimicrobial effectiveness.

This study evaluated the antimicrobial activity against food pathogens of the methanolic and ethanolic extracts of yerba mate (*Ilex paraguariensis* St. Hil.) used to prepare the typical hot mate-based beverage *chimarrão* and its relation to the content of phenolic compounds. The composition of the extract with the highest antimicrobial activity and phenolic contents was determined by gas chromatography with mass spectrometry (GC-MS) and its antimicrobial activity was evaluated under different pH conditions.

Yerba mate was acquired from the local trade in Campinas, São Paulo, Brazil. The extracts were obtained by percolation. The sample (1:8 w/v) was extracted in hydroethanolic (40:60) and hydromethanolic (30:70) solutions (Synth®, Diadema, Brazil) and maintained under refrigeration for 96 h, with filtering using qualitative filter paper 12.5 μm (Qualy®) every 24 h. The extract was evaporated in a rotary evaporator at 45 °C (Tecnal®). Then, the final extract was freeze-dried (Liotop®L101) and kept under refrigeration. For the tests, the extracts were dissolved in tryptic soy broth (TSB) (Difco®, Franklin Lakes, USA).

Antimicrobial activity from the strain collection of the Laboratório de Higiene e Laticínios (ESALQ/USP), was evaluated against *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 07644, *Salmonella* Enteritidis ATCC 13076 and *Escherichia coli* ATCC 25922. All antimicrobial tests were performed in triplicate. Antimicrobial screening was performed by agar diffusion[6]. Two hundred microliters of standardized inoculum ($1x10^8$ CFU/ml) of each organism were transferred to 200 ml of TSB plus 0.7% bacteriological agar (final population of $1.5\ x10^5$ CFU/ml). Seventy milliliters of this preparation were transferred to Petri dishes, in which 8 mm diameter wells were produced by vacuum pump and 40 μl of extracts were distributed (100 mg/ml). Negative (40 μl of TSB) and positive control (40 μl of chlorhexidine 0.12% v/v) were tested.

For MIC determination, the macrobroth dilution method was performed in 96-well microplate[3]. The extract concentrations were obtained by 2-fold serial dilution in the microplate, ranging from 25 mg/ml to 0.78 mg/ml after the addition of inoculated TSB ($1\text{-}2\ x\ 10^5$ CFU/ml). The final volume for each well was 200 μl. Positive (200 μl of TSB added of 0.12% chlorhexidine v/v) and negative control (200 μl of sterile TSB) were tested. Two hundred microliters of sterile TSB were used for broth sterility control. After incubation (35 °C/24 h), all wells received 30 μl of resazurin (0.01% w/v) (Sigma-Aldrich®, St. Louis, USA) in order to detect bacterial growth in the wells. Any evidence of color change was considered to be bacterial growth. For MBC determination, 10 μl of broth were removed from the wells considered inhibitory and sown in tryptic soy agar (TSA) (35 °C/24 h). MBC was considered as the lowest concentration at which no growth of colonies on the culture medium surface was observed.

The effectiveness of the yerba mate extract showing the best antimicrobial activity was evaluated at pH 6, 7 and 8 using 96-well microplates[9]. All wells received 100 μl of sterile TSB. One hundred μl of extracts were added into the first row of each column and then 2-fold serial dilution was performed (final concentrations ranging from 25 mg/ml to 0.78 mg/ml), after adding 100 μl of inoculated broth ($1\text{-}2\ x\ 10^5$ CFU/ml). Control groups: 200 μl of inoculated TSB (negative), 200 μl of inoculated TSB added of chlorhexidine 0.12% v/v (positive) and 200 μl of sterile TSB plus extract (white). The microplates