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Short communication

Aqueous extracts of yerba mate as bactericidal agents against methicillin-resistant *Staphylococcus aureus* in a microbiological medium and ground beef mixtures

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ABSTRACT

Antimicrobials have been routinely used in food animal production for the prevention of disease as well as for growth enhancement. Recent reports demonstrating the contamination of retail meat and poultry with multi-drug resistant bacteria have drawn public concern. Staphylococci are important opportunist pathogens affecting human and animal health. The most significant species are *Staphylococcus aureus*, which have evolved methicillin resistance. While the best method of combating these organisms is considered to be the use of non-beta-lactam antibiotics, concerns remain that these bacteria will evolve resistance to all currently used antibiotics. We present an alternative source for a natural methicillin-resistant *S. aureus* (MRSA) antimicrobial: tea, more specifically, aqueous extracts from a tea plant *Ilex paraguarensis*, yerba mate. Dialyzed, lyophilized aqueous extracts from commercially available yerba mate tea (brand Taragui, Argentina) were produced. Extracts were screened for antimicrobial activity against MRSA in a microbiological medium, tryptic soy broth (TSB), and ground beef with 7%, 15% and 27% fat content. Methicillin-resistant staphylococci were completely inactivated after 24 h incubation at 37 °C at 4 mg/ml in TSB, 16 mg/ml in 93% lean ground beef, and 32 mg/ml in both 85% and 73% lean ground beef. Lower concentrations of extracts demonstrated inactivation of MRSA following 48 h incubation: 2 mg/ml in TSB, 16 mg/ml in both 93% and 85% lean ground beef and 32 mg/ml in 73% lean ground beef. Higher concentrations of yerba mate extracts were required to inactivate MRSA in ground beef samples with higher fat content. Our results demonstrated that relatively low concentrations of yerba mate aqueous extracts provided antimicrobial activity against MRSA in ground beef. It was concluded that natural aqueous extracts derived from yerba mate have the potential to be used as natural antimicrobials against methicillin-resistant staphylococci in foods of animal origin.

1. Introduction

Staphylococci are important opportunist pathogens affecting human and animal health. The most significant species relevant to humans is *Staphylococcus aureus*, which has evolved resistance to methicillin (MRSA). Methicillin resistance in MRSA is conferred by the *mecA* gene, which encodes for the production of the penicillin-binding protein 2A, a protein with a low affinity for beta-lactams (Ubukata, Yamashita, & Konno, 1985; Utsui & Yokota, 1985; Murakami, Nomura, Doi, & Yoshida, 1987; Ubukata, Nonoguchi, Matsuhashi, & Konno, 1989). Approximately 25–30% of humans are colonized with staphylococci, with <2% colonized by MRSA (Gorwitz et al., 2008). MRSA infections are the sixth leading cause of death in hospitalized humans with a cost estimated between $4 billion and $6 billion each year (Boyce et al., 2005; Klein, Smith, & Laxminarayan, 2007). While MRSA infection has typically been identified as a problem in healthcare settings, MRSA is an emerging foodborne pathogen. Retail meat and poultry were found to be frequently contaminated with multidrug-resistant *S. aureus* (37–77%), with 52% of the *S. aureus* isolates being multidrug-resistant and 96% resistant to at least one antimicrobial (Waters et al., 2011). Additionally, there is a great risk for animal-to-human transmission of MRSA, recently reported in Denmark pig farmers (Lewis et al., 2008). Similar to methicillin-sensitive strains of *S. aureus*, MRSA strains also have the ability to produce enterotoxins, which can cause food intoxication if consumed. Such an outbreak occurred in the United States and was traced to MRSA-contaminated shredded pork barbeque and coleslaw prepared by a person harboring MRSA (Jones, Kellum, Porter, Bell, & Schaffner, 2002).

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The diversity of multi-drug resistant bacteria is apparently increasing, which adds to the clinical management problems wherein effective antibiotics are limited. In addition, there is a trend to use natural products as preservatives and antimicrobials. Many plants and their extracts have been used in the discovery and development of novel antimicrobial agents [reviewed in (Cowan, 1999; Sampedro & Valdivia, 2014)]. Yerba mate tea, which is produced from the leaves and stems of the shrub plant *Ilex paraguarensis*, is a popularly consumed beverage in the South American countries of Brazil, Uruguay, Paraguay and Argentina. This plant has been extensively studied for a variety of pharmacological properties—antioxidant (Anesini, Ferraro, & Filip, 2006; Bastos, 2007; Bastos, Ishimoto, Marques, Ferri, & Torres, 2006; Bastos et al., 2007; Carini, Facino, Aldini, Calloni, & Colombo, 1998; Filip, Lotito, Ferraro, & Fraga, 2000; Gugliucci & Stahl, 1995; Pagliosa et al., 2010), antiobesity (Andersen & Fogh, 2001), antidiabetic (Lunceford & Gugliucci, 2005), diuretic (Gorgen et al., 2005), chemopreventative, antifungal (Filip, Davicino, & Anesini, 2010), stimulant (Athayde, Coelho, & Schenkel, 2000; Filip, Lopez, Coussio, & Ferraro, 1998), digestive aid (Gorzalczany et al., 2001), probiotic (Gonzalez-Gil et al., 2014), and recently for its antimicrobial activity against several common foodborne pathogens (Burris, Davidson, Stewart, & Harte, 2011; Burris, Davidson, Stewart, Zivanovic, & Harte, 2012; Hongpattarakere, 2000, p. 189), including *Escherichia coli* O157:H7 (Burris et al., 2011; Burris, Davidson, et al., 2012; Hongpattarakere, 2000, p. 189) and methicillin-susceptible *S. aureus* (Burris et al., 2011; Burris, Davidson, et al., 2012). Additionally, yerba mate extracts have demonstrated antimicrobial activity against several other Gram-positive bacteria, *Listeria monocytogenes, Bacillus subtilis,* (Hongpattarakere, 2000, p. 189; Kubo, Muroi, & Himejima, 1993), *Brevibacterium ammoniagenes* (Kubo et al., 1993), and *Streptococcus mutans* (Kubo et al., 1993) as well as against the Gram-negative bacteria, *Salmonella* Typhimurium and *Pseudomonas fluorescens* (Hongpattarakere, 2000, p. 189; Kubo et al., 1993; Sari, Turkmen, Polat, & Velioglu, 2007; Tsai, Tsai, Chien, Lee, & Tsai, 2008).

These pharmacological properties of yerba mate may be attributed to several identified compounds from its extracts, including xanthines, caffeoyl derivatives, saponins, and minerals (Alikaridis, 1987; Bastos, 2007; Bastos et al., 2006, 2007; Bravo, Goya, & Lecumberri, 2007; Cardozo Jr et al., 2007; Carini et al., 1998; Clifford & Ramirezmartinez, 1990; Filip, Lopez, Giberti, Coussio, & Ferraro, 2001; Gosmann & Schenkel, 1989; Heck & de Mejia, 2007; Marques & Farah, 2009). The minimum bactericidal concentration (MBC) of an aqueous yerba mate extract against the Gram-negative foodborne pathogen *E. coli* O157:H7 was determined to be 5 mg/ml for strain ATCC 43894 and 10 mg/ml for strain 'Cider' in a microbiological medium (Burris, Davidson, et al., 2012). Hongpattarakere (2000, p. 189) found that yerba mate extracts from water, methanol, acetonitrile, ethanol, ethyl acetate, isopropanol, chloroform, butanol, dichloromethane, petroleum ether, and methanol:water (4:1) demonstrated some level of antimicrobial activity against *S. aureus*. However, greatest inhibition was observed with water, methanol and methanol:water (4:1) extracts (Hongpattarakere, 2000, p. 189).

The U.S. Food and Drug Administration (FDA) values the decreased use of antibiotics for use in food animal production and has moved toward increasing their regulation for that purpose. Thus, there is a dire need for alternative compounds to control multi-drug resistant bacteria. In this study, we examined the effectiveness of aqueous extracts of commercially-available yerba mate tea in a microbiological medium at 1, 2, 4, 8 mg/ml and in 93%, 85% and 73% lean ground beef at 4, 8, 16, and 32 mg/ml against two strains of MRSA. We evaluated the anti-MRSA activities of these yerba mate extracts after 0, 3, 6, 9, 24 and 48 h at 37 °C to assess the efficacy of a potential prophylaxis of a foodborne pathogen contamination.

2. Materials and methods

2.1. Aqueous extraction

Dried leaves of a single commercial brand of yerba mate tea (Taragui; Argentina; *Ilex paraguariensis*) were purchased from a local international supermarket. Extracts were obtained by using previous methods (Burris, Davidson, et al., 2012) with modifications as described herein. Briefly, dried leaves were finely ground to a particle size of less than 300 μm using a commercial blender (Oster, Boca Raton, Florida, USA). Sterile deionized water was added to ground leaves at a ratio of 3.6 ml to 1 g ground tissue, were allowed to stand for 2 h at 4 °C with occasional mixing to maximize extraction and were subsequently centrifuged at 5000 × *g* for 30 min. Aqueous extracts were then dialyzed at 4 °C against deionized water for 36 h using a 3500 MWCO SnakeSkin® pleated dialysis tubing (Thermo-Fisher Scientific, Rockford, Ill., USA) to remove low molecular weight compounds. Dialyzed extracts were centrifuged at 5000 × *g* for 30 min to remove large insoluble particles and were frozen at −80 °C. Frozen extracts were then lyophilized using Labconco FreeZone 12 L Freeze Dry System (Labconco, Kansas City, Missouri, USA) to concentrate them. Lyophilized extracts were stored at room temperature in a sealed container until testing.

2.2. Phenolic content determination

Lyophilized extract (50 mg) was rehydrated in 50 ml deionized water to a final concentration of 1 mg/ml, filtered through Whatman No. 4 paper and analyzed for total phenolic content. Phenolic content was quantified spectrophotometrically at 765 nm using Folin-Ciocalteu's phenol reagent (Montreau, 1972) with gallic acid as the standard and results expressed in mg gallic acid equivalents (GAE)/g dry extract. Results were calculated as the mean value of three replications ± standard error.

2.3. Culture preparation

MRSA strains ATCC 33591 and ATCC 33593 were purchased from American Type Culture Collection (ATCC; Manassas, Virginia, USA). Bacteria were selected on Baird-Parker medium (Becton, Dickinson and Company, Sparks, Maryland, USA) and stock cultures were prepared by isolating a single colony, growing in tryptic soy broth (TSB; Becton, Dickinson and Company) and stored at −20 °C in glycerol. Working cultures were attained by inoculating 50 ml TSB with 200 μl stock cultures, incubating for 24 h at 35–37 °C, and were subcultured at least once. Following incubation, ca. 9.0 $\log_{10}$ CFU/ml cultures were diluted to ca. 6.0 $\log_{10}$ CFU/ml and tested for antimicrobial activity.

2.4. Time kill assays

Lyophilized extracts (0–800 mg) were diluted in 10 ml sterile water and filter sterilized using 0.22 μm (Millipore), mixed with bacteria harvested from cultures grown overnight and diluted (initial bacterial count of ca. 9.0 $\log_{10}$ CFU/ml diluted to a final concentration of ca. 6.0 $\log_{10}$ CFU/ml). Ground beef mixtures (10% w/v) were made by combining 30 g of commercially obtained ground beef (93%, 85% or 73% lean ground beef; Kroger, Inc., Knoxville, Tenn., USA) with 300 ml tryptic soy broth (TSB, Becton, Dickinson and Company) and sterilized by autoclaving for 30 min. A total volume of 25 ml was used, which consisted of 12.5 ml of TSB or TSB ground beef mixture (10% w/v), 10 ml of filtered extracts or sterile deionized water (positive control), and 2.5 ml of inoculum or 0.1% peptone (negative control). Bacteria and extracts were incubated in TSB or TSB ground beef mixture (10% w/v) at 35–37 °C and at regular intervals (0, 3, 6, 9, 24 and 48 h) a bacterial suspension

sample (0.1 or 1 ml) was collected, serially diluted in 0.1% peptone, plated in duplicate (0.1 or 1 ml) using tryptic soy agar (TSA; TSB, Becton, Dickinson and Company, and agar, Fisher Scientific, Fair Lawn, N.J., USA), incubated for 24 h at 35–37 °C, and then enumerated (expressed as $\log_{10}$ CFU/ml). All treatments were plated in duplicate, each experiment was repeated at least twice, and average values ($\log_{10}$ CFU/ml) were reported.

2.5. Statistical analysis

A completely randomized design was used. All experiments were replicated at least twice and each treatment was plated in duplicate for analysis. Data were analyzed by analysis of variance (ANOVA) using the general linear model (SAS 9.4, SAS Institute, Cary, N.C., USA). Least significant differences (LSD) were used to compare treatment mean values when significant differences ($P < 0.05$) were found. Error bars represent 95% confidence intervals for the mean using LSD.

3. Results

For every 100 g of ground yerba mate tea (particle size less than 300 μm, brand Taragui, Argentina) used, approximately 4–5 g lyophilized extract was acquired. Phenolic content was determined to be approximately 77 mg GAE/g lyophilized extract.

Yerba mate extracts at all concentrations tested were effective at inactivating MRSA ATCC 33591 and ATCC 33593 to undetectable levels in microbiological medium after 24 h, an approximate 6.0 $\log_{10}$ CFU/ml reduction (Fig. 1). Complete inhibition of MRSA ATCC 33591 and ATCC 33593 to undetectable levels occurred after only 12 h at 8 mg/ml and 4 mg/ml respectively (Fig. 1).

Inactivation to undetectable levels (ca. 6.0 $\log_{10}$ CFU/ml reduction) of a 1:1 mixture of MRSA strains ATCC 33591 and ATCC 33593 was observed at 4 mg/ml in TSB, 16 mg/ml in 7% fat ground beef mixture and 32 mg/ml in 15% fat and 27% fat ground beef mixtures after 24 h incubation and at 2 mg/ml in TSB, 16 mg/ml in 7% and 15% fat ground beef and 32 mg/ml in 27% fat ground beef after 48 h (Fig. 2). The higher the fat content of the ground beef mixtures, the greater the concentration of yerba mate extract was required to inactivate MRSA.

4. Discussion

In the present study, extracts from a commercial tea, yerba mate, were examined for their effectiveness at inhibiting and/or inactivating MRSA in microbiological medium and in ground beef mixtures with varying fat content. Yerba mate is known to contain numerous bioactive compounds including polyphenols, xanthines, caffeoyl derivatives, saponins, and minerals (reviewed in (Bastos, 2007; Burris, Harte, Davidson, Stewart, & Zivanovic, 2012; Heck & de Mejia, 2007)). The most common compounds of the hydroxycinnamic acid family found in plants are caffeic, ferulic, sinapic, and *p*-coumaric acids (Bastos, 2007). Several purified forms of these compounds identified from yerba mate yielded promising antimicrobial results against a variety of Gram-positive and Gram-negative bacteria (Chakraborty & Mitra, 2008; Herald & Davidson, 1983; Panizzi, Caponi, Catalano, Cioni, & Morelli, 2002; Rauha et al., 2000). However, much of the literature is conflicted with regards to which compound(s) contribute to antimicrobial activity. Ravn, Andary, Kovacs, and Mogaard (1989) determined that caffeic acid derivatives were effective at inhibiting *S. aureus*; however found that pure caffeic acid did not demonstrate activity against *S. aureus* or two strains of *Staphylococcus epidermidis*. In agreement with Ravn et al. (1989), Herald and Davidson (1983) demonstrated a reduction in viable *S. aureus* at pH 5.0 by *p*-coumaric acid, a hydroxycinnamic acid. Caffeoylquinic acid derivatives have been identified from yerba mate extracts (Filip et al., 2010, 2000; Jaiswal, Sovdat, Vivan, & Kuhnert, 2010) and have demonstrated anti microbial activity in other crude plant extracts (Chakraborty & Mitra, 2008). Thus, these compounds may contribute to the antimicrobial activity of yerba mate against methicillin-resistant staphylococci.

The flavonols, kaempferol, quercetin and rutin, have also been identified from yerba mate and in their pure forms have been studied for antimicrobial activity (Panizzi et al., 2002; Rauha et al., 2000). It was found by Rauha et al. (2000) that kaempferol did not inhibit *S. epidermidis*, but was antimicrobial against *S. aureus*. Like kaempferol, quercetin exhibited strong inhibition against *S. aureus*, but also provided strong to moderate activity against *S. epidermidis* (Rauha et al., 2000). In contrast, Panizzi et al. (2002) determined that neither kaempferol nor quercetin provided antimicrobial activity against *S. aureus*. The compound rutin provided no antimicrobial activity against either *S. aureus* or *S. epidermidis* (Rauha et al., 2000). Similar to the results found by Panizzi et al. (2002), kaempferol, quercetin, rutin nor *p*-coumaric acid provided antimicrobial activity against six MRSA strains tested (Otsuka et al., 2008). Thus, there seems to be evidence that the flavonols may not be responsible for the anti-staphylococcal activity of yerba mate.

While much research has been performed on pure compounds identified from yerba mate and other plant extracts, identification of the exact compound or compounds responsible for antimicrobial activity is still unknown. It is likely that a combination of known and/or unidentified compounds found in yerba mate extracts is responsible for its antimicrobial activity against staphylococci. Additionally, the compound(s) responsible for antimicrobial activity appear to be quite stable, having gone through the relatively harsh processing steps of blanching, drying, milling and aging for commercial preparation. It is also possible that the compound is generated during one of the processing steps. A comparative HPLC quantitative analysis of samples collected during various stages of commercial processing demonstrated that a higher content of biologically active components was observed following zapecado, drying and aging steps as compared with green leaves (Isolabella et al., 2010). While we have not fully identified the compound(s) contributing to its antimicrobial activity, crude extracts and several isolated compounds derived from yerba mate have been shown active against a broad spectrum of Gram-positive and Gram-negative bacteria (Burris et al., 2011; Burris, Harte, et al., 2012; Hongpattarakere, 2000, p. 189; Kubo et al., 1993; Prado Martin et al., 2013; Sari et al., 2007; Tsai et al., 2008), thus demonstrating its potential to be used as a novel antimicrobial in foods or animal production to combat methicillin-resistant staphylococci or other foodborne pathogenic bacteria. Ethanolic and methanolic extracts of yerba mate were shown inhibitory to *S. aureus, L. monocytogenes, and S. Enteritidis*, with activity attributed to the presence of compounds derived from chlorogenic acid (Prado Martin et al., 2013).

It is common that relatively high concentrations of antimicrobials are required for activity when used in complex food systems, such as beverages and meats, than for microbiological media to reach the same level of bacterial inactivation. In our previous study, 4–8 times higher concentrations (40 mg/ml) of yerba mate extract were required to decrease *E. coli* O157:H7 ca. 4.5 $\log_{10}$ CFU/ml in pH-adjusted apple juice than in microbiological medium (5–10 mg/ml) (Burris, Harte, et al., 2012). In our present study, higher concentrations of yerba mate (4–16 times higher) were required to inactivate *S. aureus* in ground beef as fat levels increased. The highest effective concentration of yerba mate required to inactivate MRSA in microbiological medium was ineffective at eliminating MRSA in ground beef mixtures. Similar observations were observed for hot dogs (Higginbotham, Burris, Zivanovic, Davidson, & Stewart, 2014b) and milk (Higginbotham, Burris, Zivanovic, Davidson, & Stewart, 2014a) treated with *Hibiscus* extract. Higher concentrations of *Hibiscus* extract demonstrated greater bactericidal activity

against MRSA when used as a hot dog wash, with counts below detection after 60 min wash and 24 h storage with the highest concentration of extract tested (240 mg/ml) (Higginbotham et al., 2014b). When *Hibiscus* extracts were tested for activity against *E. coli* O157:H7 and *S. aureus* in skim (<0.5%), 1%, 2%, and whole (>3.25%) milk, extracts were less effective in 1%, 2% and whole milk than skim against *E. coli.* However, at all fat levels tested, extracts were equally inhibitory to *S. aureus.*

5. Conclusions

Yerba mate extracts were shown to inactivate or inhibit MRSA at relatively low concentrations (2–32 mg/ml) in ground beef mixtures, depending on fat level. To our knowledge, this is the first report demonstrating the antimicrobial activity of extracts from yerba mate against MRSA in ground beef. The results presented here indicate that the natural aqueous extracts derived from yerba mate may be used as a natural alternative to chemical antimicrobials against MRSA in food animal production as well as for the potential development of a new compound to treat staphylococcal infections from MRSA in humans and animals, potentially reducing incidences of MRSA in retail meat products. Further research needs to be conducted to demonstrate the antimicrobial effects of these extracts in other retail meat products as well as perform sensory analysis to determine consumer acceptability. Ultimately, a comprehensive investigation into the identification of the potential compound(s) providing activity would be necessary to evaluate their toxicological risk for use as additives to foods, although yerba mate is currently generally recognized as safe (GRAS) for both animals (21 CFR 582.20) and humans (21 CFR 182.20) by the Food and Drug Administration (FDA).

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Appendix

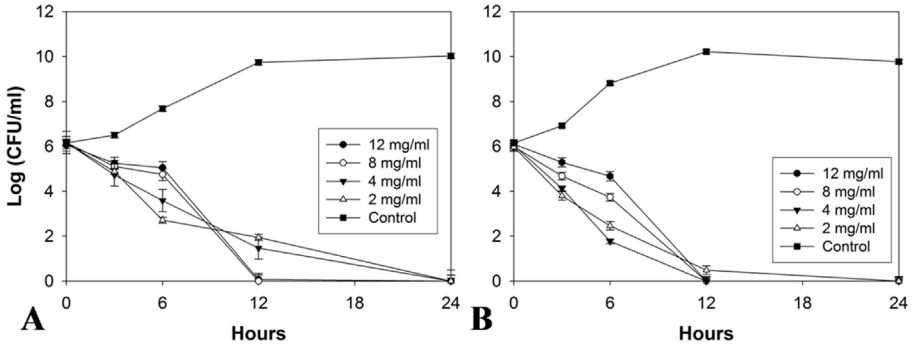

Fig. 1. Antimicrobial activity of yerba mate (brand Taragui, Argentina) extracts at 0, 2, 4, 8 and 12 mg/ml against methicillin-resistant *Staphylococcus aureus* (A) ATCC 33591 and (B) ATCC 33593 in a microbiological medium. Error bars represent 95% confidence intervals using least significant differences ($P < 0.05$).

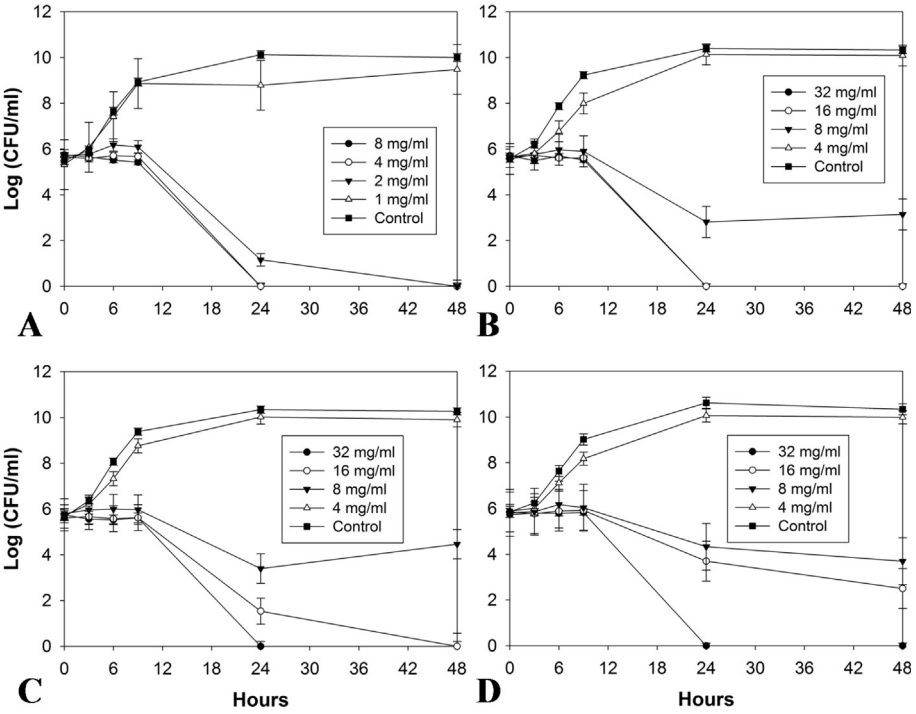

Fig. 2. Antimicrobial activity of yerba mate (brand Taragui, Argentina) extracts against a 1:1 mixture of methicillin-resistant *Staphylococcus aureus* strains ATCC 33591 and ATCC 33593 at (A) 0, 1, 2, 4 and 8 mg/ml in tryptic soy broth, (B) 0, 4, 8, 16, and 32 mg/ml in 93% lean ground beef, (C) 0, 4, 8, 16, and 32 mg/ml in 85% lean ground beef, and (D) 0, 4, 8, 16, and 32 mg/ml in 73% lean ground beef. Error bars represent 95% confidence intervals for the mean using least significant differences ($P < 0.05$).

References

Alikaridis, F. (1987). Natural constituents of *Ilex* species. *Journal of Ethnopharmacology, 20*(2), 121–144.

Andersen, T., & Fogh, J. (2001). Weight loss and delayed gastric emptying following a South American herbal preparation in overweight patients. *Journal of Human Nutrition and Dietetics, 14*(3), 243–250.

Anesini, C., Ferraro, G., & Filip, R. (2006). Peroxidase-like activity of *Ilex paraguariensis*. *Food Chemistry, 97*(3), 459–464.

Athayde, M. L., Coelho, G. C., & Schenkel, E. P. (2000). Caffeine and theobromine in epicuticular wax of *Ilex paraguariensis* A St -Hil. *Phytochemistry, 55*(7), 853–857.

Bastos, D. H. (2007). Pharmacological properties, research and biotechnology. *Medicinal and Aromatic Plant Science and Biotechnology, 1*, 37–46.

Bastos, D. H. M., Ishimoto, E. Y., Marques, M. O. M., Ferri, A. F., & Torres, E. A. F. S. (2006). Essential oil and antioxidant activity of green mate and mate tea (*Ilex paraguariensis*) infusions. *Journal of Food Composition and Analysis, 19*(6–7), 538–543.

Bastos, D. H. M., Saldanha, L. A., Catharino, R. R., Sawaya, A. C. H. F., Cunha, I. B. S., Carvalho, P. O., et al. (2007). Phenolic antioxidants identified by ESI-MS from yerba mate (*Ilex paraguariensis*) and green tea (*Camelia sinensis*) extracts. *Molecules, 12*(3), 423–432.

Boyce, J. M., Cookson, B., Christiansen, K., Hori, S., Vuopio-Varkila, J., Kocagoz, S., et al. (2005). Meticillin-resistant *Staphylococcus aureus*. *The Lancet Infectious Diseases, 5*(10), 653–663.

Bravo, L., Goya, L., & Lecumberri, E. (2007). LC/MS characterization of phenolic constituents of mate (*Ilex paraguariensis*, St. Hil.) and its antioxidant activity compared to commonly consumed beverages. *Food Research International, 40*(3), 393–405.

Burris, K. P., Davidson, P. M., Stewart, C. N., Jr., & Harte, F. M. (2011). Antimicrobial activity of yerba mate (*Ilex paraguariensis*) aqueous extracts against *Escherichia coli* O157:H7 and *Staphylococcus aureus*. *Journal of Food Science, 76*(6), M456–M462.

Burris, K. P., Davidson, P. M., Stewart, C. N., Jr., Zivanovic, S., & Harte, F. M. (2012). Aqueous extracts of yerba mate (*Ilex paraguariensis*) as a natural antimicrobial against *Escherichia coli* O157:H7 in a microbiological medium and pH 6.0 apple juice. *Journal of Food Protection, 75*(4), 753–757.

Burris, K. P., Harte, F. M., Davidson, P. M., Stewart, C. N., Jr., & Zivanovic, S. (2012). Composition and bioactive properties of yerba mate (*Ilex paraguariensis* A. St.-Hil.): a review. *Chilean Journal of Agricultural Research, 72*(2), 268–274.

Cardozo, E. L., Jr., Ferrarese-Filho, O., Filho, L. C., Ferrarese, M.d. L. L., Donaduzzi, C. M., & Sturion, J. A. (2007). Methylxanthines and phenolic compounds in mate (*Ilex paraguariensis* St. Hil.) progenies grown in Brazil. *Journal of Food Composition and Analysis, 20*(7), 553–558.

Carini, M., Facino, R. M., Aldini, G., Calloni, M., & Colombo, L. (1998). Characterization of phenolic antioxidants from mate (*Ilex paraguayensis*) by liquid chromatography mass spectrometry and liquid chromatography tandem mass spectrometry. *Rapid Communications in Mass Spectrometry, 12*(22), 1813–1819.

Chakraborty, M., & Mitra, A. (2008). The antioxidant and antimicrobial properties of the methanolic extract from *Cocos nucifera* mesocarp. *Food Chemistry, 107*(3), 994–999.

Clifford, M. N., & Ramirezmartinez, J. R. (1990). Chlorogenic acids and purine alkaloids contents of mate (*Ilex paraguariensis*) leaf and beverage. *Food Chemistry, 35*(1), 13–21.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*(4), 564–582.

Filip, R., Davicino, R., & Anesini, C. (2010). Antifungal activity of the aqueous extract of *Ilex paraguariensis* against *Malassezia furfur*. *Phytotherapy Research, 24*(5), 715–719.

Filip, R., Lopez, P., Coussio, J., & Ferraro, G. (1998). Mate substitutes or adulterants: study of xanthine content. *Phytotherapy Research, 12*(2), 129–131.

Filip, R., Lopez, P., Giberti, G., Coussio, J., & Ferraro, G. (2001). Phenolic compounds in seven South American *Ilex* species. *Fitoterapia, 72*(7), 774–778.

Filip, R., Lotito, S. B., Ferraro, G., & Fraga, C. G. (2000). Antioxidant activity of *Ilex paraguariensis* and related species. *Nutrition Research, 20*(10), 1437–1446.

Gonzalez-Gil, F., Diaz-Sanchez, S., Pendleton, S., Andino, A., Zhang, N., Yard, C., et al. (2014). Yerba mate enhances probiotic bacteria growth in vitro but as a feed additive does not reduce *Salmonella* Enteritidis colonization *in vivo*. *Poultry Science, 93*(2), 434–440.

Gorgen, M., Turatti, K., Medeiros, A. R., Buffon, A., Bonan, C. D., Sarkis, J. J. F., et al. (2005). Aqueous extract of *Ilex paraguariensis* decreases nucleotide hydrolysis in rat blood serum. *Journal of Ethnopharmacology, 97*(1), 73–77.

Gorwitz, R. J., Kruszon-Moran, D., McAllister, S. K., McQuillan, G., McDougal, L. K., Fosheim, G. E., et al. (2008). Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001-2004. *Journal of Infectious Diseases, 197*(9), 1226–1234.

Gorzalczany, S., Filip, R., Alonso, M. D., Mino, J., Ferraro, G. E., & Acevedo, C. (2001). Choleretic effect and intestinal propulsion of 'mate' (*Ilex paraguariensis*) and its substitutes or adulterants. *Journal of Ethnopharmacology, 75*(2–3), 291–294.

Gosmann, G., & Schenkel, E. P. (1989). A new saponin from mate, Ilex paraguariensis. *Journal of Natural Products, 52*(6), 1367–1370.

Gugliucci, A., & Stahl, A. J. C. (1995). Low-density-lipoprotein oxidation is inhibited by extracts of *Ilex paraguariensis*. *Biochemistry and Molecular Biology International, 35*(1), 47–56.

Heck, C. I., & de Mejia, E. G. (2007). Yerba mate tea (*Ilex paraguariensis*): a comprehensive review on chemistry, health implications, and technological considerations. *Journal of Food Science, 72*(9), R138–R151.

Herald, P. J., & Davidson, P. M. (1983). Anti-bacterial activity of selected hydroxycinnamic acids. *Journal of Food Science, 48*(4), 1378–1379.

Higginbotham, K. L., Burris, K. P., Zivanovic, S., Davidson, P. M., & Stewart, C. N., Jr. (2014a). Antimicrobial activity of *Hibiscus sabdariffa* aqueous extracts against *Escherichia coli* O157:H7 and *Staphylococcus aureus* in a microbiological medium and milk of various fat concentrations. *Journal of Food Protection, 77*(2), 262–268.

Higginbotham, K. L., Burris, K. P., Zivanovic, S., Davidson, P. M., & Stewart, C. N., Jr. (2014b). Aqueous extracts of *Hibiscus sabdariffa* calyces as an antimicrobial rinse on hot dogs against *Listeria monocytogenes* and methicillin-resistant *Staphylococcus aureus*. *Food Control, 40*, 274–277.

Hongpattarakere, T. (2000). *Natural antimicrobial components isolated from yerba mate (Ilex paraguariensis) leaves* (DPhil dissertation). Madison, WI: University of Wisconsin.

Isolabella, S., Cogoi, L., Lopez, P., Anesini, C., Ferraro, G., & Filip, R. (2010). Study of the bioactive compounds variation during yerba mate (*Ilex paraguariensis*) processing. *Food Chemistry, 122*(3), 695–699.

Jaiswal, R., Sovdat, T., Vivan, F., & Kuhnert, N. (2010). Profiling and characterization by LC-MSn of the chlorogenic acids and hydroxycinnamoylshikimate esters in mate (*Ilex paraguariensis*). *Journal of Agricultural and Food Chemistry, 58*(9), 5471–5484.

Jones, T. F., Kellum, M. E., Porter, S. S., Bell, M., & Schaffner, W. (2002). An outbreak of community-acquired foodborne illness caused by methicillin-resistant *Staphylococcus aureus*. *Emerging Infectious Diseases, 8*(1), 82–84.

Klein, E., Smith, D. L., & Laxminarayan, R. (2007). Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999-2005. *Emerging Infectious Diseases, 13*(12), 1840–1846.

Kubo, I., Muroi, H., & Himejima, M. (1993). Antibacterial activity against *Streptococcus mutans* of mate tea flavor components. *Journal of Agricultural and Food Chemistry, 41*(1), 107–111.

Lewis, H. C., Molbak, K., Reese, C., Aarestrup, F. M., Selchau, M., Sorum, M., et al. (2008). Pigs as source of methicillin-resistant CC398 infections in humans, Denmark. *Emerging Infectious Diseases, 14*(9), 1383–1389.

Lunceford, N., & Gugliucci, A. (2005). *Ilex paraguariensis* extracts inhibit AGE formation more efficiently than green tea. *Fitoterapia, 76*(5), 419–427.

Marques, V., & Farah, A. (2009). Chlorogenic acids and related compounds in medicinal plants and infusions. *Food Chemistry, 113*(4), 1370–1376.

Montreau, F. (1972). On the analysis of total phenolic compounds in wines by the Folin–Ciocalteau method. *Connaissance de la vigne et du vin, 24*, 397–404.

Murakami, K., Nomura, K., Doi, M., & Yoshida, T. (1987). Production of low-affinity penicillin-binding protein by low- and high-resistance groups of methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy, 31*, 1307–1311.

Otsuka, N., Liu, M. H., Shiota, S., Ogawa, W., Kuroda, T., Hatano, T., et al. (2008). Anti-methicillin resistant *Staphylococcus aureus* (MRSA) compounds isolated from *Laurus nobilis*. *Biological and Pharmaceutical Bulletin, 31*(9), 1794–1797.

Pagliosa, C. M., Vieira, M. A., Podestá, R., Maraschin, M., Zeni, A. L. B., Amante, E. R., et al. (2010). Methylxanthines, phenolic composition, and antioxidant activity of bark from residues from mate tree harvesting (*Ilex paraguariensis* A. St. Hil.). *Food Chemistry, 122*(1), 173–178.

Panizzi, L., Caponi, C., Catalano, S., Cioni, P. L., & Morelli, I. (2002). *In vitro* antimicrobial activity of extracts and isolated constituents of *Rubus ulmifolius*. *Journal of Ethnopharmacology, 79*(2), 165–168.

Prado Martin, J. G., Porto, E., de Alencar, S. M., da Glória, E. M., Corrêa, C. B., & Ribeiro Cabral, I. S. (2013). Antimicrobial activity of yerba mate (*Ilex paraguariensis* St. Hil.) against food pathogens. *Revista Argentina de Microbiología, 45*(2), 93–98.

Rauha, J. P., Remes, S., Heinonen, M., Hopia, A., Kahkonen, M., Kujala, T., et al. (2000). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology, 56*(1), 3–12.

Ravn, H., Andary, C., Kovacs, G., & Mogaard, P. (1989). Caffeic acid esters as *in vitro* inhibitors of plant pathogenic bacteria and fungi. *Biochemical Systematics and Ecology, 17*(3), 175–184.

Sampedro, J., & Valdivia, E. (2014). New antimicrobial agents of plant origin. In T. G. Villa, & P. Veiga-Crespo (Eds.), *Antimicrobial compounds* (pp. 83–114). Springer Berlin Heidelberg.

Sari, F., Turkmen, N., Polat, G., & Velioglu, Y. S. (2007). Total polyphenol, antioxidant and antibacterial activities of black mate tea. *Food Science and Technology Research, 13*(3), 265–269.

Tsai, T. H., Tsai, T. H., Chien, Y. C., Lee, C. W., & Tsai, P. J. (2008). *In vitro* antimicrobial activities against cariogenic streptococci and their antioxidant capacities: a comparative study of green tea versus different herbs. *Food Chemistry, 110*(4), 859–864.

Ubukata, K., Yamashita, N., & Konno, M. (1985). Occurrence of a b-lactam-inducible penicillin-binding protein in methicillin resistant staphylococci. *Antimicrobial Agents and Chemotherapy, 27*, 851–857.

Ubukata, K., Nonoguchi, R., Matsuhashi, M., & Konno, M. (1989). Expression and inducibility in *Staphylococcus aureus* of the *mecA* gene, which encodes a methicillin-resistant *S. aureus*-specific penicillin-binding protein. *Journal of Bacteriology, 171*(5), 2882–2885.

Utsui, Y., & Yokota, T. (1985). Role of an altered penicillin binding protein in methicillin- and cephem-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy, 28*, 397–404.

Waters, A. E., Contente-Cuomo, T., Buchhagen, J., Liu, C. M., Watson, L., Pearce, K., et al. (2011). Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. *Clinical Infectious Diseases, 52*(10), 1227–1230.