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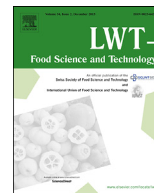

Arabica coffee extract shows antibacterial activity against *Staphylococcus epidermidis* and *Enterococcus faecalis* and low toxicity towards a human cell line

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ABSTRACT

The antimicrobial activity of a regular and decaffeinated Arabica coffee extract was evaluated against three different Gram-positive bacteria and two Gram-negatives, including pathogenic *Staphylococci* strains. The antimicrobial activity was shown to be independent from caffeine content and was more pronounced against the Gram-positive strains. The regular coffee extract exhibited a significant bacteriostatic effect against *Staphylococcus aureus* and *Staphylococcus epidermidis* at short exposure times and became bactericidal after prolonged exposure. The potential cytotoxicity of the regular coffee extract was also evaluated towards breast adenocarcinoma MCF7 cells, showing to become significant only after 24 h exposure and at a higher concentration than that producing the antibacterial effect. These results highlight the potential of coffee extracts as a naturally active and non-toxic antibacterial compound suitable for biomedical applications.

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1. Introduction

The increase in the antimicrobial resistance in bacterial populations raises the question of an urgent response in terms of new antimicrobial molecules (Zell & Goldmann, 2007). However the development of new antibiotic molecules takes a long time scale and is expensive both in terms of human and financial resources, therefore the preferred strategy is to optimize already existing antimicrobial drugs or to combine multiple antibiotic compounds to improve their antimicrobial potency. Furthermore, the overwhelming concern of the society over the safety of the synthetic molecules has lead to an increased interest towards molecules of natural origin.

Some studies have reported antimicrobial property for coffee (Almeida, Farah, Silva, Nunan, & Gloria, 2006; Daglia et al., 2007; Rurian-Henares & Morales, 2008; Tiwari et al., 2009), however the antibacterial components responsible for the activity and the mechanisms of action have not been fully elucidated yet (Mueller, Sauer, Weigel, Pichner, & Pischetsrieder, 2011). Previous studies with *Staphylococcus aureus* ATCC25923 and *Escherichia coli* ATCC25922 showed that the antimicrobial potential of coffee is related to the roasting procedure and is dependent on the degree of roasting, focusing the attention on the products of the Maillard reaction as potentially responsible for the observed antimicrobial activity (Daglia, Cuzzoni, & Dacarro, 1994). In line with this hypothesis, antimicrobial activities of melanoidins isolated from coffee have been reported (Rurian-Henares & Morales, 2008). However, due to the extremely variable composition of these molecules, derived from carbohydrates, proteins/amino acids and phenolic compounds formed during the roasting procedure (Bekedam, Loots, Schols, Van Boekel, & Smit, 2008), the exact mechanism by which the antimicrobial effect takes place remains difficult to elucidate.

Abbreviations: MH, Mueller-Hinton; CQAs, caffeoylquinic acids; FQAs, feruoylquinic acids; UHPLC, Ultra High Performance Liquid Chromatography; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; FIC, fractional inhibitory concentration; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; PBS, phosphate buffered saline; MTT, 1-(4, 5-Dimethylthiazol-2-yl)-3, 5-diphenylformazan; MRSA, methicillin-resistant *Staphylococcus aureus*.

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Rurian-Henares and Morales showed that a coffee fraction, corresponding to high molecular weight molecules such as melanoidins, was able to disrupt at the minimum inhibitory concentration both the inner and outer membrane of an *E. coli* strain, leading to the release of intracellular molecules (Rurian-Henares & Morales, 2008). Moreover, metal chelating properties were proposed as an important feature to mediate the antibacterial activity of coffee (Rufian-Henares & de la Cueva, 2009).

For future use of coffee derivatives as antibacterial compounds it is important to unravel the mechanisms mediating their antimicrobial properties and also to broaden the number of tested strains to define their spectrum of activity. In this respect, *Staphylococcus epidermidis* and *Enterococcus faecalis* are two bacterial species which have not been extensively tested in the past. The first is the dominant species among the resident flora on hands (Rayan & Flournoy, 1987) and is one of the two most frequent cause of nosocomial infection, together with *S. aureus* (National Nosocomial Infections Surveillance System, 2004). On the other hand *E. faecalis* is responsible of nosocomial infections such as urinary tract and abdominal infections, bacteremia and endocarditis in patients with severe concomitant diseases or with an impaired immune system (Mundy, Sahm, & Gilmore, 2000).

Less is known about the potential cytotoxicity of coffee extracts against eukaryotic cells and tissues. Hegele et al. showed that the major cytotoxic component in Maillard reaction mixtures and coffee (prepared as filter coffee and espresso) is represented by hydrogen peroxide which is formed through an autooxidative process where polyphenolics reduce atmospheric oxygen in the presence of transition metals (Hegele, Munch, & Pischetsrieder, 2009). To our knowledge no other data are available.

The aim of the present study was to investigate the antimicrobial activity and cytotoxicity of Arabica coffee extracts. Bacterial species such as *S. epidermidis* and *E. faecalis*, which have not been assayed yet, and an eukaryotic cell line have been assayed with the extracts. Results contribute to evaluate the potentiality of these coffee derivatives as antimicrobials or preservatives.

2. Materials and methods

2.1. Bacterial strains, media and growth conditions

The bacterial strains used were from the American Type Culture Collection (ATCC) and included the three Gram-positive cocci *S. aureus* ATCC25923, *S. epidermidis* ATCC12228 and *E. faecalis* ATCC29212, and the two Gram-negative bacilli *E. coli* ATCC25922 and *Salmonella enterica* ATCC14028. Bacterial cultures were grown in Mueller-Hinton (MH) broth (Difco Laboratories, Becton Dickinson and Company, Sparks, MD) at 37 °C under aerobic conditions.

2.2. Preparation of the regular and decaffeinated total coffee extracts

Regular and decaffeinated coffee extracts were prepared starting from 6 g coffee powder (100% Arabica, medium roasted), previously defatted by extraction with penthane, by solid–liquid extraction under continuous stirring with 100 ml boiling water 10 min at 100 °C (Daglia et al., 2007). The aqueous extracts were centrifuged 10 min at 1600 × *g* and the clear supernatant was then filtered through 0.45 μm filter and subsequently through 0.22 μm filter. Aliquots of the obtained coffee extracts were freeze-dried and stored at −20 °C until used. The average extraction yields were around 25% (w/w).

2.3. Chemicals

Caffeine, trigonelline, formic acid, acetonitrile and methanol were purchased from Sigma–Aldrich (Steinheim am Albuch, Germany); 5-, 4- and 3-caffeoylquinic acid (CQAs) and 3,4- 3,5- and 4,5-dicaffeoylquinic acid were purchased from Phytolab GmbH & Co. KG (Vestenbergsgreuth, Germany); ferruoylquinic acids (FQAs) were obtained from the division of organic chemistry and biochemistry at Ruder Bošković Institute (Zagreb, Croatia) (Dokli, Navarini, & Hamersak, 2013). Numbering of substituted position on CQAs and FQAs was designated according to the IUPAC system. Water was purified on a Milli-Q system from Millipore (Bedford, MA).

2.4. UHPLC analysis of the coffee extracts

The analysis was performed using a 1290 UHPLC system (Agilent, Waldbronn, Germany), consisting of a degasser, quaternary pump, thermostated column and diode array detector (DAD) operating at 254 nm, 272 nm and 324 nm. Samples were prepared by dissolving the lyophilized powder in a solution of 60% (v/v) methanol in Milli-Q water and then filtered through a 0.22 μm filter. 2 μl of the sample were injected in the UHPLC system and the flow rate was 1.2 ml/min. For caffeine, trigonelline and 3-, 4-, 5-chlorogenic acid, determinations were carried out using a 4.6 mm × 150 mm, 2.7 μm 120 SB-C18 Poroshell column (Agilent, Santa Clara, CA) and a gradient elution (acetonitrile and 0.1% (v/v) formic acid). For minor compounds as ferruoylquinic acids, a 75 mm × 4.6 mm, 2.6 μm Kinetex Phenyl Hexyl column equipped with SecurityGuard™ Ultra cartridges for Phenyl UHPLC (Phenomenex, Torrance, CA) and a similar elution gradient were used. Identification and quantitation of compounds were performed by external calibration of standard compounds on a 5-points calibration curve.

Confirmation of the caffeine content in the regular and decaffeinated extracts was performed via the ISO 20481:2008 reference method, using a 1100 HPLC system (Agilent, Waldbronn, Germany) equipped with a 4.6 mm × 150 mm, 5 μm MS-C18 XTerra column, isocratic elution of water/methanol 76/24, 10 μl injection volume and 272 nm detection wavelength.

2.5. Antimicrobial activity assays for caffeine and for the regular and decaffeinated coffee extracts

For the preparation of the stock solution of the regular and decaffeinated coffee extracts the lyophilized powder was resuspended in sterile Milli-Q water at a final concentration ranging from 100 to 350 mg/ml for all the antimicrobial activity assays. For the caffeine stock a solution was prepared dissolving 150 mg of caffeine powder (Sigma–Aldrich, St. Louis, MO) in 1 ml sterile Milli-Q water at 80 °C.

Minimum inhibitory concentration (MIC) values of the regular and decaffeinated coffee extracts and of caffeine were determined using the broth microdilution susceptibility test following the guidelines of the NCCLS with mid-log phase cultures. Serial two-fold dilutions of each extract or of caffeine were prepared in a final volume of 50 μl in 96-well polystyrene plates (Sarstedt, Nümbrecht, Germany) with MH broth. Each dilution series included control wells without the extract or without caffeine. A volume of 50 μl of a bacterial suspension at a concentration of 5×10^5 cells/ml was then added to each well. The MIC was taken as the lowest concentration of regular and decaffeinated coffee extract or of caffeine resulting in the complete inhibition of visible growth after 20 h of incubation at 37 °C. For the determination of the minimum bactericidal concentration (MBC) 25 μl of broth from clear wells were spotted in triplicate on an MH agar plate which

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