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

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Running title: Antibacterial activity and cytotoxicity of coffee extracts

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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 a coffee extract as a naturally active antibacterial component for topical use such as for hand washing preparations to be used in health care units.

Keywords: Arabica coffee extract, antimicrobial activity, cytotoxicity

Abbreviations

MH, Mueller-Hinton broth; CQAs, caffeoylquinic acids; FQAs, ferruoylquinic 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 S. aureus.
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* ATCC 25923 and *Escherichia coli* ATCC 25922 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.

Rufián-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).

To extend the use of coffee derivatives as antibacterial compounds for topical use, as active components of hand washing preparations, it is essential to unravel the mechanisms mediating their antimicrobial properties and 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 *Staphylococcus 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).

Contrary to what is observed for the antimicrobial effects of coffee extracts, less is known about its potential cytotoxicity. 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).

The aim of the present study was to investigate the antimicrobial activity of Arabica coffee extracts against bacterial species such as *Staphylococcus epidermidis* and *Enterococcus faecalis*, which have not been extensively studied in the past, and to assay possible cytotoxicity of the extracts towards eukaryotic cells. Results are useful to evaluate the potentiality of the extracts as antimicrobials to be used in topical preparations.
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 Staphylococcus aureus ATCC25923, Staphylococcus epidermidis ATCC12228 and Enterococcus faecalis 29212, and the two Gram-negative bacilli Escherichia 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 pentane, by solid-liquid extraction under continuous stirring with 100 ml boiling water 10 min at 100°C. 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 (Vestenbergsreuth, 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 feruloylquinic 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 and 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 $5 \times 10^5$ 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 a MH agar plate which was then incubated 20 h at 37°C. MBC was defined as the lowest concentration of coffee extract killing at least 99.99% of the original inoculum.

To monitor bacterial growth inhibition, a bacterial suspension of $1 \times 10^6$ cells/ml was grown in a 96-well polystyrene plate (Sarstedt, Nümbrecht, Germany) with periodic shaking at 37°C in presence of 5 mg/ml coffee extract for *E. coli*, *S. enterica* and *E. faecalis* or in presence of 0.5 mg/ml for *S. aureus* and *S. epidermidis*. The OD$_{620}$ was measured on a microtiter plate reader (Tecan Trading AD, Männedorf, Switzerland) every 10 min for *E. coli* and *S. enterica* or every 30 min for *E. faecalis*, *S. aureus* and *S. epidermidis*.

For the viable colony count a bacterial suspension of $1 \times 10^6$ cells/ml was grown with shaking at 37°C in presence of 1, 2, 4 mg/ml coffee extract for *S. aureus* and *S. epidermidis* or in presence of 5 mg/ml coffee extract for *E. faecalis*. At each time point (1, 2, 4 h) an aliquot of the sample was serially diluted in fresh MH broth and 25 μl of the serial dilutions were spotted in triplicate on a MH agar plate which was then incubated 20 h at 37°C to allow the viable colony count.
2.5.1. Antimicrobial activity of the regular coffee extract in combination with vancomycin

The activity of the regular coffee extract combined to vancomycin (Sigma-Aldrich, St. Louis, MO) was evaluated by the checkerboard technique in a 96-well polystyrene plate (Euroclone, Milan, Italy). Briefly, concentrations of vancomycin, ranging from 8 μM (12 μg/ml) to 0.125 μM (0.18 μg/ml), and regular coffee extract, ranging from 6 mg/ml to 0.09 mg/ml for *S. aureus* and *S. epidermidis* or from 60 mg/ml to 0.93 mg/ml for *E. faecalis*, were combined in the standard MIC format along with a bacterial suspension at a concentration of $5 \times 10^5$ cells/ml. The microplate was incubated 20 h at 37°C and then the fractional inhibitory concentration (FIC) index was calculated for each combination as follows: FIC index = Σ (FIC\textsubscript{A} + FIC\textsubscript{B}), where FIC\textsubscript{A} is the MIC of compound A in combination/MIC of compound A alone, and FIC\textsubscript{B} is MIC of compound B in combination/MIC of compound B alone. Synergy was defined for a FIC index of ≤ 0.5, indifference for a FIC index of > 0.5 to ≤ 4, and antagonism for a FIC index of > 4 (Eliopoulos, 1996).

2.6. Cytotoxicity towards eukaryotic cells

The human breast carcinoma cell line was purchased from the ECACC N°86012803 (MCF7) and cultured in Dulbecco’s modified Eagle’s (DMEM) High Glucose medium (Euroclone, Milan, Italy) supplemented with 10% (v/v) Fetal Bovine Serum (FBS) (Euroclone, Milan, Italy), 2 mM L-glutamine (Euroclone, Milan, Italy) and penicillin (100 U/ml)-streptomycin (100 μg/ml) (Sigma-Aldrich, St. Louis, MO).

For the cytotoxicity assay, the MCF7 cells were seeded at a density of 20,000 cells per well, in a volume of 100 μl, in a 96-well polystyrene plate (Euroclone, Milan, Italy) and incubated at 37°C with 5% CO\(_2\). After 1 h, 2 h, 4 h, 24 h exposure to different extract concentrations (1, 2, 3, 4 mg/ml) in supplemented DMEM High Glucose medium, the adherent cells were washed with Phosphate Buffered Saline (PBS) (Sigma-Aldrich, St. Louis, MO) and 20 μl of 5 mg/ml 1-(4, 5-Dimethylthiazol-2-yl)-3, 5-diphenylformazan (MTT) (Sigma-Aldrich, St. Louis, MO) were added to 100 μl supplemented DMEM High Glucose medium in each well. After 4 h incubation at 37°C
with 5% CO₂, the culture medium supernatant was removed and the formazan was dissolved with 10% (w/v) tert-octylphenoxy poly(oxyethylene) ethanol (IGEPAL) (Sigma-Aldrich, St. Louis, MO) in HCl 0.01 N overnight at 37°C. The absorbance at 620 nm was measured on a microtiter plate reader (Tecan Trading AD, Männedorf, Switzerland).

For the 23 h recovery in fresh DMEM High Glucose medium after 1 h exposure to the extract, the cells were washed with PBS and then 100 μl of fresh medium were added to each well. Afterwards the microplate was incubated at 37°C with 5% CO₂ for the additional 23 h and then MTT was added as described above.

2.7. Statistical analysis

The significance of differences among bacterial strains treated with increasing concentrations of coffee extract for the viable colony count was assessed using GraphPad Prism (GraphPad Software, La Jolla, CA) by the ANOVA multiple comparison test with Student-Newman-Keuls post test for the Staphylococci strains and by the Unpaired t-test for the E. faecalis strain. For the cytotoxicity assay towards breast adenocarcinoma MCF7 cells the significance of differences among the various treatments was assessed using GraphPad Prism by the ANOVA multiple comparison test with Student-Newman-Keuls post test.

3. Results and Discussion

3.1. Analysis of the regular and decaffeinated coffee extracts

The relative content of trigonelline, 3-, 4-, 5-CQAs, 3,4-, 3,5-, 4,5-diCQAs and 3-, 4-, 5-FQAs of the regular and of the decaffeinated coffee extracts was determined by UHPLC-DAD. Results indicate that the concentrations of all these components did not differ significantly between the two coffee extracts and that the only difference was the relative content of caffeine (Table 1), which was almost undetectable in the decaffeinated coffee extract. As expected, the specificity of the solvent
used in the decaffeination process resulted in a remarkable decrease in the caffeine content only (Farah, de Paulis, Moreira, Trugo, & Martin, 2006) and this permits to put in evidence the role played by caffeine which is not yet fully disclosed (Antonio et al., 2010; Almeida et al., 2012).

3.2. Antimicrobial activity of the regular and decaffeinated coffee extracts

The antimicrobial activity of a regular Arabica coffee extract was measured against three Gram-positive and two Gram-negative bacterial strains. The results are expressed as the MIC values for the tested strains (Table 2) and showed that the extract was active against all the strains. As a general result Gram-positive bacteria showed a higher susceptibility to the extracts with respect to the Gram-negative ones. The only exception was represented by *E. faecalis* which showed a MIC value similar to that of the Gram-negative strains (Table 2). Results obtained with *S. aureus* and *E. faecalis* are in agreement with previous data obtained using brewed coffee samples (Daglia, et al., 1994; Rufian-Henares & de la Cueva, 2009). Excluding *E. faecalis*, the overall higher sensitivity of the Gram-positive bacteria towards the extract could be due to the different composition of the bacterial cell envelope in Gram-negative and positive bacteria, the latter lacking a low-permeability barrier such as the outer membrane.

To evaluate the role of caffeine in the antimicrobial activity of the regular coffee extract, a decaffeinated coffee extract (see Materials and methods) was also tested in parallel with pure caffeine. The results showed that there are no differences between regular and decaffeinated Arabica coffee extracts in the ability to inhibit the bacterial growth (Table 2). Furthermore the MIC values for pure caffeine are much higher for all the tested bacterial strains than the caffeine concentration determined in the regular coffee extract at the MIC value, which is < 0.1 mg/ml (Table 1). These results are in agreement with those obtained by Manzhu Kang et al. who reported that in presence of 4 mg/ml caffeine *E. coli* failed to grow (Kang et al., 2012).

These results highlight the predominant antibacterial effect of components other than caffeine in the extract. Indeed Daglia et al. pointed to α-dicarbonyl compounds as the main agents responsible for
the antibacterial activity of roasted coffee against \textit{Staphylococcus aureus} and \textit{Streptococcus mutans} (Daglia, et al., 2007). Moreover, other roasted coffee constituents including melanoidins (the final products of the Maillard reaction), polyphenols, trigonelline and both caffeic and protocatechuic acids have shown to exert antibacterial activity (Morales, Somoza, & Fogliano, 2012) Almeida et al., 2012).

We then investigated the ability of the regular coffee extract to inhibit the bacterial growth even at concentrations lower than the MIC values (Figure 1). In presence of 0.3-0.5 \(\times\) MIC values of the regular coffee extract all the tested bacterial strains showed a decreased growth rate. However the highest inhibition of the bacterial growth was observed for \textit{E. faecalis}: the effect of the extract on the growth rate of this strain was significantly higher than that observed for \textit{E. coli} or \textit{S. enterica}, even though the MIC value was comparable for the three strains.

To evaluate whether the extract has a bacteriostatic effect, we studied the short-term effect of the extract against \textit{S. aureus} and \textit{S. epidermidis}, which showed the highest susceptibility to the extract in terms of MIC values, and against \textit{E. faecalis} to better understand the effect of the extract on the growth rate of this strain.

Against both \textit{Staphylococci} strains the extract showed a bacteriostatic effect which was appreciable already after 1 h incubation and became more pronounced after prolonged incubation time at all the tested concentrations, leading to a 2-log growth reduction after 4 h incubation at the maximum concentration tested (4 mg/ml) for both \textit{S. aureus} and \textit{S. epidermidis} (Figure 2). These results suggest that the antibacterial activity of the regular coffee extract is mainly bacteriostatic at short exposure time and becomes bactericidal only after prolonged incubation. To confirm this observation the MBC values were determined for both \textit{S. aureus} and \textit{S. epidermidis}. We found that the MBC correspond to the MIC values of the extract for each strain (data not shown), indicating that after prolonged incubation (20 h) the extract was able to kill bacteria. The observation that the extract becomes bactericidal only after prolonged incubation time might suggest that it has a non-
lytic mechanism of action, which in contrast would have shown a strong and rapid bactericidal effect. However further studies will be required to clarify this point.

On the opposite, the treatment of *E. faecalis* with 5 mg/ml extract produced a significant bacteriostatic effect only after 4 h exposure to the extract (Figure 2B). This result, taken together with the inhibition of the growth rate showed in Figure 1B, suggests that the extract exhibits a delayed effect on this bacterial strain, beginning to significantly slow the bacterial growth and to affect the bacterial viability only after 4 h treatment.

To test a potential synergic effect of the regular coffee extract in combination with an antibiotic often used to treat nosocomial severe methicillin-resistant *S. aureus* (MRSA) infections (Rayner & Munckhof, 2005), the antimicrobial activity of the extract was evaluated in combination with vancomycin.

Given that vancomycin acts by inhibiting proper cell wall synthesis in Gram-positive bacteria, we investigated whether the regular coffee extract enhanced its activity. The FIC indexes showed that all combinations of regular coffee extract plus vancomycin did not exhibit any synergic effect against *S. aureus* nor *S. epidermidis* (0.5 < FIC index ≤ 4). Similar results were obtained also for the combination of regular coffee extract plus vancomycin against *E. faecalis* (0.5 < FIC index ≤ 4), suggesting that this antibiotic and the extract act independently one from each other.

### 3.3. Cytotoxicity effect of the regular coffee extract

The eventual toxicity of the regular coffee extract was assayed towards breast adenocarcinoma MCF7 cells (Figure 3), a cell line that is used as a model for cytotoxic studies of chemotherapeutic agents. At a 3 mg/ml concentration, corresponding to 1.5- and 3-fold the MIC value for *S. aureus* and *S. epidermidis* respectively, no significant toxicity was observed up to 4 h exposure to the extract (Figure 3A). At this concentration, a significant decrease in cell viability was detected only with a 24-h exposure. We further investigated the cytotoxicity of the extract after prolonged exposure time showing that until 1 mg/ml the extract does not exhibit any effect on the viability of...
the cells even after 24 h incubation (Figure 3B), while at 2 and 4 mg/ml the extract showed a significant toxicity towards the MCF7 cells. However, when the cells were left for 23 h in fresh medium, after the 1-h exposure, no toxicity was observed at any of the tested concentrations (Figure 3C), suggesting that, even if a damage might be produced by the extract after 1 h exposure, the cells are able to repair it during the next 23 h.

Hydrogen peroxide, which in coffee is mainly formed by roasting products, was already shown to play an important role in the cytotoxicity of coffee extracts towards bovine aorta endothelial cells (Hegele, et al., 2009). This molecule might have a role also in cytotoxicity towards MCF7 cells and further experiments will be needed to clarify this mechanism.

These data are encouraging considering that after 2 h exposure to the minimum concentration of coffee extract (1 mg/ml) a clear antibacterial effect is already appreciable for both *S. aureus* and *S. epidermidis*, while the toxicity towards the MCF7 cells begins to be significant only after 24 h exposure to a concentration which is 4-fold (4 mg/ml) that producing a significant bacteriostatic effect.

**4. Conclusions**

In conclusion, we showed that an Arabica regular coffee extract has a wide-spectrum antibacterial activity. The extract was effective against *S. epidermidis*, which represent the dominant species among the resident flora on hands, and has been shown to act as a reservoir for the transfer of genetic elements to enhance the pathogenicity of *S. aureus* (Diep et al., 2006). The two *Staphilococci* strains are often responsible of nosocomial infections resistant to the antibiotics commonly used. In this respect we propose the use of the regular coffee extract as a component for topical preparations to be used in healthcare units aimed at reducing the transmission of pathogens by hand contact. Furthermore the observed antimicrobial activity of the extract against *E. faecalis* and *S. aureus* might be promising also for its use as a food preservative. Indeed both bacterial species may be present in many food products and can cause toxinfestions (Giraffa, 2002;
Hennekinne, De Buyser, & Dragacci, 2012). Overall the kinetics of activity of the coffee extract appear to be slower than that of other antimicrobials, with the extract showing mainly a bacteriostatic effect at short exposure times, a feature that may be useful to arrest growth of contaminant bacteria in food.

Finally, we showed that the Arabica coffee extract at the antibacterial concentrations does not cause significant cytotoxic effects towards a model cell line. Therefore we believe that this kind of compound deserves to be further studied with the aim to develop antibacterials for topical use, as active components of hand washing preparations.

Acknowledgments

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References


Figure captions

Figure 1. Growth kinetics of different Gram-negative (A) and Gram-positive (B) bacterial strains in presence of the regular coffee extract. Bacterial suspensions of $10^6$ cells ml$^{-1}$ were grown for 4 h (A) or 8 h (B) in absence (solid line) or in presence (dashed line) of the extract and the OD$_{620}$ was recorded every 10 min (A) or 30 min (B). Results are the mean of three independent experiments ± SD.

Figure 2. Viable colony counts of the Gram-positive strains exposed to the regular coffee extract. Bacteria were incubated for different hours in presence of different concentrations of the extract, serially diluted with MH Broth, and plated to allow the viable colony counts. The results are reported as CFU/ml (log scale) and represent the mean of three independent experiments ± SD. (A) * p<0.05, ** p<0.01, *** p<0.001 (ANOVA with Student-Newman-Keuls post test). (B) * p<0.05 (Unpaired t-test).

Figure 3. Cytotoxicity towards breast adenocarcinoma MCF7 cells. (A) Percentage of viable cells compared to the control (no coffee extract, 100%) after increasing incubation times with 3 mg/ml extract (grey bars). (B) Percentage of viable cells compared to the control (no coffee extract, 100%) after 24 h exposure. (C) Percentage of viable cells compared to the control (no coffee extract, 100%) after 1 h exposure and 23 h recovery in fresh medium. Results represent the mean ± SEM of at least two independent experiments each performed in triplicate. ** p<0.01, *** p<0.001 vs untreated control (ANOVA with Student-Newman-Keuls post test).
Table 1. Relative content of trigonelline, caffeine, chlorogenic and dichlorogenic acids, and ferruylquinic acids in coffee extracts.

<table>
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<th>regular (mg/g)</th>
<th>decaffeinated (mg/g)</th>
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<td>trigonelline</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>caffeine</td>
<td>38</td>
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<td>1</td>
</tr>
<tr>
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<td>11</td>
</tr>
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<td>21</td>
<td>22</td>
</tr>
<tr>
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<td>0.3</td>
</tr>
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<td>0.1</td>
</tr>
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</tr>
<tr>
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<tr>
<td>5-FQA</td>
<td>0.6</td>
<td>0.9</td>
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<sup>a</sup>Determined by the ISO 20481:2008 reference method.
Table 2. Sensitivity of different bacteria to total aqueous extracts obtained from Arabica coffee and to pure caffeine.

<table>
<thead>
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<th></th>
<th>MIC (mg/ml)\textsuperscript{a}</th>
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<tr>
<td></td>
<td>regular</td>
</tr>
<tr>
<td>\textit{S. aureus} ATCC25923</td>
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<td>\textit{S. epidermidis} ATCC12228</td>
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<tr>
<td>\textit{E. faecalis} ATCC29212</td>
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<tr>
<td>\textit{S. enterica} ATCC14028</td>
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</tbody>
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\textsuperscript{a}Results are representative of three independent experiments.
Figure 1

A

E. coli

MH Broth + 5 mg/ml coffee extract

S. enterica

MH Broth + 0.5 mg/ml coffee extract

S. aureus

MH Broth + 0.5 mg/ml coffee extract

E. faecalis

MH Broth + 5 mg/ml coffee extract

B

S. epidermidis

MH Broth + 0.5 mg/ml coffee extract

S. aureus

MH Broth + 0.5 mg/ml coffee extract

E. faecalis

MH Broth + 5 mg/ml coffee extract
Figure 2

A

S. aureus

B

E. faecalis

S. epidermidis

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untreated + 1 mg/ml extract + 2 mg/ml coffee extract + 4 mg/ml coffee extract

untreated + 5 mg/ml extract

untreated + 1 mg/ml extract + 2 mg/ml coffee extract + 4 mg/ml coffee extract

untreated + 1 mg/ml coffee extract
Figure 3

A

B

C

Viable cells (%)

coffee extract (mg/ml)

time (h)

Viable cells (%)

coffee extract (mg/ml)

Viable cells (%)

coffee extract (mg/ml)