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# Coriander (*Coriandrum sativum* L.) essential oil: its antibacterial activity and mode of action evaluated by flow cytometry

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**Abbreviations:** BOX, bis-1,3-dibutylbarbutiric acid; CTC, 5-cyano-2,3-ditolyl tetrazolium chloride; EB, ethidium bromide; FSC, forward scatter; MBC, minimum bactericidal concentration; PI, propidium iodide; SSC, side scatter.

The aim of this work was to study the antibacterial effect of coriander (*Coriandrum sativum*) essential oil against Gram-positive and Gram-negative bacteria. Antibacterial susceptibility was evaluated using classical microbiological techniques concomitantly with the use of flow cytometry for the evaluation of cellular physiology. Our results showed that coriander oil has an effective antimicrobial activity against all bacteria tested. Also, coriander oil exhibited bactericidal activity against almost all bacteria tested, with the exception of *Bacillus cereus* and *Enterococcus faecalis*. Propidium iodide incorporation and concomitant loss of all other cellular functions such as efflux activity, respiratory activity and membrane potential seem to suggest that the primary mechanism of action of coriander oil is membrane damage, which leads to cell death. The results obtained herein further encourage the use of coriander oil in antibacterial formulations due to the fact that coriander oil effectively kills pathogenic bacteria related to foodborne diseases and hospital infections.

## Introduction

Essential oils of herbs and their components, which are products from the secondary metabolism of plants, have many applications in ethno-medicine, food flavouring and preservation as well as in the

fragrance and pharmaceutical industries (Fabian *et al.*, 2006). The antimicrobial properties of essential oils have been described (Pinto *et al.*, 2006, 2009) and, because of the growing demand on antimicrobials for preventing microbial food spoilage and bacterial infections, there is an increasing interest in medicinal plants as an alternative to synthetic preservatives and antibiotics (Edris, 2007). Many essential oils are already used in the food industry as flavouring agents and some are known to exert antimicrobial activity, but the mechanism of action is often not entirely understood.

Coriander (*Coriandrum sativum* L.) is a well-known herb widely used as a spice, in folk medicine and in the pharmacy and food industries (Burdock & Carabin, 2009). Coriander seed oil is one of the 20 major essential oils in the world market (Lawrence, 1993) and it is known to exert antimicrobial activity (Burdock & Carabin, 2009); however, its mechanism of action is still unclear.

In this work, we assessed the antimicrobial action of *C. sativum* essential oil against Gram-positive and Gram-negative bacteria. We describe the use of flow cytometry to investigate the effects of coriander oil on membrane and cellular functions, which were evaluated using several fluorochromes: propidium iodide (PI) for membrane integrity, bis-1,3-dibutylbarbutiric acid (BOX) for membrane potential, ethidium bromide (EB) for efflux activity and 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) for respiratory activity.

## Methods

### Bacterial strains.

Antibacterial activity of commercial coriander oil extracted by steam distillation (Sigma-Aldrich) was tested against 12 bacterial strains of which seven were reference strains: four Gram-negative reference strains (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhimurium* ATCC 13311 and *Pseudomonas aeruginosa* ATCC 27853), two clinical multidrug-resistant *Acinetobacter baumannii* isolates (*A. baumannii* 2/10 and *A. baumannii* 3/10), three Gram-positive reference strains (*B. cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212), one clinical *S. aureus* isolate (*S. aureus* SA08) and two clinical meticillin-resistant *S. aureus* isolates (MRSA 10/08 and MRSA 12/08).

The bacterial isolates were identified by standard microbiology methods. All bacterial strains were stored in Brain Heart Infusion (BHI) broth with 20% (v/v) glycerol at −80 °C. Prior to susceptibility testing, each strain was inoculated on BHI agar to ensure optimal growth and purity.

### Determination of MIC and minimum bactericidal concentration (MBC).

A microdilution broth susceptibility assay for bacteria was used, as recommended by the Clinical and Laboratory Standards Institute for the determination of the MIC (NCCLS, 2003). The MBC was determined according to the NCCLS (1999) guidelines. All tests were performed in Mueller–Hinton broth (MHB) supplemented with DMSO (maximum final concentration of 2%, v/v) to

enhance the oil solubility, with the exception of *E. faecalis* MIC and MBC determination, where BHI broth was used to facilitate growth visualization. Test strains were suspended in 0.8% (w/v) NaCl to obtain a cell density of $1–2\times10^8$ c.f.u. $ml^{-1}$. These cell suspensions were diluted to obtain a cell density of $1\times10^6$ c.f.u. $ml^{-1}$ and were further diluted to obtain a final density of $5\times10^5$ c.f.u. $ml^{-1}$ in each well. Geometric dilutions ranging from 0.025 to 3.2% (v/v) of the essential oil were prepared in a 96-well microtitre plate. Growth conditions (MHB with 2%, v/v, DMSO with tested micro-organism) and sterility of the medium (MHB) were checked. Oil sterility was checked by substituting the inoculum with MHB, or BHI in the case of *E. faecalis*, and performing the oil serial dilutions previously described. The plates were incubated under normal atmospheric conditions at 37 °C for 24 h. The MIC was defined as the lowest concentration of oil which prevented visible growth. The MBC was defined as the lowest concentration of oil where 99.9% or more of the initial inoculum was killed and was determined by subculture of 20 µl, from each well where visible growth was prevented, on agar plates. Each experiment was repeated at least three times at each test concentration and the modal MIC and MBC values were selected.

**Exposure of bacteria to coriander oil.**

Suspensions of the micro-organisms were obtained from an exponentially growing culture of each strain in BHI broth at 37 °C and 250 r.p.m. Cultures were then centrifuged at 10000 ***g*** for 5 min, washed twice in buffered peptone water and resuspended in the same. This cell suspension was used to inoculate tubes containing the appropriate concentrations of coriander oil (MIC and MBC values) and 2% (v/v) DMSO in peptone water to obtain a cell density of about $1–2\times10^7$ c.f.u. $ml^{-1}$. The inoculated tubes were incubated at 37 °C and 250 r.p.m. For the cases in which the MIC value was equal to the MBC value, cells were incubated with an oil concentration of twice the MIC value. The contact time established for flow cytometric experiments was 30 min, since higher contact times up to 4 h yielded similar results. At the end of 30 min, samples were centrifuged at 10000 ***g*** for 15 min, washed and resuspended in 1 ml sterile PBS.

In all cases, control experiments were carried out in parallel; cells were incubated in buffered peptone water either with or without 2% (v/v) DMSO and treated under the same conditions.

**Bacterial cell counts.**

Serial dilutions of samples exposed to coriander oil were prepared in sterile 0.85% (w/v) NaCl solution and plated according to the method previously described (Chen *et al.*, 2003). The plates were incubated at 37 °C for 24 h, and the number of colonies was determined. To measure growth inhibition, viability reduction was calculated from these data according to Bouhdid *et al.* (2009), where $N_C$ is the bacterial count in control sample without DMSO.

**Staining procedures for flow cytometry experiments.**

For membrane permeability assessment, suspensions of Gram-positive and Gram-negative cells ($1\times10^6$ c.f.u. $ml^{-1}$) were incubated with 10 μg PI $ml^{-1}$ and 1 μg PI $ml^{-1}$ (Sigma-Aldrich) in PBS buffer (pH 7.4), respectively. Gram-positive bacterial suspensions were incubated for 5 min at room temperature and Gram-negative bacteria were incubated for 15 min at 37 °C.

For the evaluation of membrane potential, suspensions of Gram-positive and Gram-negative cells ($1\times10^6$ c.f.u. $ml^{-1}$) were incubated with 0.5 μg BOX $ml^{-1}$ and 2.5 μg BOX $ml^{-1}$ (Molecular Probes, Invitrogen, part of Life Technologies) in PBS buffer (pH 7.4) and PBS buffer with 4 mM EDTA (pH 7.4), respectively. Gram-positive suspensions were incubated for 2 min at room temperature and Gram-negative bacteria were incubated for 15 min at 37 °C.

In the case of efflux activity staining, suspensions of Gram-positive and Gram-negative cells ($1\times10^6$ c.f.u. $ml^{-1}$) were incubated with 5 μg EB $ml^{-1}$ and 10 μg EB $ml^{-1}$ (Sigma-Aldrich) in PBS buffer with 1% (w/v) glucose (pH 7.4) and PBS buffer (pH 7.4), respectively. Gram-positive bacterial suspensions were incubated for 5 min at room temperature and Gram-negative bacteria were incubated for 15 min at 37 °C.

In order to assess respiratory activity, suspensions of Gram-positive and Gram-negative cells ($1\times10^6$ c.f.u. $ml^{-1}$) were incubated with 5 mM CTC (Polysciences) in PBS buffer with 1% (w/v) glucose (pH 7.4) and incubated for 30 min at 37 °C and 250 r.p.m.

Ethanol-fixed cells (Silva *et al.*, 2010) were used as a positive control for PI, BOX and EB staining protocols and as a negative control for CTC staining. Cells incubated in buffered peptone water without 2% (v/v) DMSO were used as negative control for PI, BOX and EB and as negative control for CTC staining. Prior to the flow cytometric analysis, all cell suspensions were washed once in PBS buffer (pH 7.4) and resuspended in the same buffer. Experiments were conducted in duplicate.

**Flow cytometry.**

Bacterial samples were analysed on a BD Biosciences FACSCalibur. Acquisition was performed with CellQuest Pro Software and based on light-scatter and fluorescence signals resulting from 15 mW laser illumination at 488 nm. Light-scatter measurements and fluorescence measurements were acquired logarithmically. Signals corresponding to forward and side scatter (FSC and SSC) and fluorescence were accumulated; the fluorescence signal (pulse area measurements) was collected by FL-1 (BOX) and FL-3 (PI, EB and CTC) bandpass filters. Threshold levels were set on SSC to eliminate noise or particles (of cellular debris) much smaller than intact cells. Bacterial cells were gated according to FSC/SSC parameters. Sample acquisition was operated at low flow rate setting (12 μl sample $min^{-1}$). A total of 10000 events were acquired for each sample. Data analysis was performed using FCS Express version 3 Research Edition (De Novo Software).

## Results and Discussion

## Antimicrobial activity of coriander oil

Coriander essential oil has been reported to inhibit a broad spectrum of micro-organisms (Delaquis *et al.*, 2002; Duman *et al.*, 2010; Lo Cantore *et al.*, 2004). Antimicrobial activity of coriander essential oil against reference and clinical strains used in this work is presented in Table 1. All strains studied were inhibited by coriander oil, with different degrees of inhibition. *B. cereus* was the most sensitive strain along with one of the multidrug-resistant clinical strains of *A. baumannii* (MIC=0.1%, v/v), while *P. aeruginosa* was the most resistant to growth inhibition by the tested oil, showing the highest determined MIC (1.6%, v/v). According to results obtained by Duman *et al.* (2010), the activity of coriander essential oil was higher than the activity of its main constituent, linalool. Furthermore, when using fractions obtained by fractional distillation of coriander essential oil, the fraction that presented as less potent but effective against tested micro-organisms was the one containing a superior concentration of linalool (Delaquis *et al.*, 2002). These findings suggest that the antimicrobial activity is due to complex interactions between individual components that lead to the overall activity and not only to the effects of linalool, as could be expected.

The bactericidal activity of this essential oil is shown by the MBC values. When comparing the MBC values for Gram-positive and Gram-negative strains, it is clear that the action of the oil is more pronounced in Gram-negative than in Gram-positive bacteria. For *B. cereus* and *E. faecalis*, the MBC value was even higher than the highest oil concentration tested (>3.2%, v/v). Gram-negative strains and *S. aureus* strains inhibited by coriander oil were found not to be able to regrow in fresh culturing medium after transfer and incubation for 24 h, indicating that this oil possesses bactericidal activity, already described for *Campylobacter jejuni* (Rattanachaikunsopon & Phumkhachorn, 2010).

Our results showed that, in general, Gram-positive bacteria are less susceptible than Gram-negative bacteria to coriander oil. Given that studies investigating the action of essential oils against bacteria are not consensual about essential oils being more active against Gram-positive or Gram-negative bacteria (Lo Cantore *et al.*, 2004; Tassou *et al.*, 1995), the effect of this oil on cellular functions must be further understood. Therefore, it was decided to investigate the oil effect on cellular functions such as respiratory activity, efflux activity, membrane potential and membrane integrity using flow cytometry.

## Membrane permeability

PI was used to assess bacterial membrane integrity because, if membrane integrity is compromised, PI can enter the cell where it binds to nucleic acids. Membrane permeability is often well correlated with bacterial counts. Overall, there was a slight reduction in bacterial viability (Tables 2 and 3) of control cells with DMSO, possibly due to DMSO effects on membrane permeability (Notman *et al.*, 2006).

In the control population with 2% DMSO, no more than 17% of cells were PI-positive; however, a higher percentage of Gram-positive cells were PI-stained compared to Gram-negative cells (Tables 2 and 3). With respect to Gram-negative strains, less than 90% of *K. pneumoniae* and *Salmonella typhimurium* cells were PI-positive, even when cells were treated with oil concentrations corresponding to MBC values (0.2 and 0.8%, respectively) (Table 3). When *P. aeruginosa* was treated with an oil concentration corresponding to the MBC value (1.6%), the viability reduction did not reach 99.9%, which was only achieved with an oil concentration of twice the MBC, though less than 90% (86.3%) of cells were PI-positive. Regarding *E. coli*, the percentage of PI-stained cells increased with oil concentration, always being higher than 90% (93.73% and 99.79%). In the case of the *A. baumannii* clinical isolates, almost 100% of cells were PI-stained with an oil concentration corresponding to the MBC value (Table 3) (96.10% and 98.09% for *A. baumannii* 02/10 and *A. baumannii* 03/10, respectively), with no visible alteration in the percentage of staining when cells were incubated with an oil concentration of twice the MBC.

With respect to Gram-positive bacteria, treated cells exhibited higher percentages of PI-stained cells, with the exception of *B. cereus* (Table 3). For the *S. aureus* strains used, almost 100% of cells were PI-stained when treated with an oil concentration corresponding to the MBC value, with viability reduction values of 99.9–100%, with slightly lower values for *S. aureus* SA08 (98.73%), which is in agreement with the high percentages of PI-positive cells obtained (Table 3). In the *S. aureus* strains, except *S. aureus* SA08 (90%), a viability reduction of 99.9% or more was also obtained for oil concentrations corresponding to the MIC. In the case of *E. faecalis* and *B. cereus*, viability reduction was lower than 99.9% (Table 3), which is in agreement with the MBC values obtained previously (Table 1). For *E. faecalis*, there was no visible alteration in the PI-positive cells when incubated with 0.8 and 3.2% oil and the percentage of PI-positive *B. cereus* cells was the lowest of all the bacteria tested (Tables 2 and 3) with no more than 55% of stained cells. The morphological alterations visualized by flow cytometry (FSC and SSC) (Fig. 1a–c) and confirmed by light microscopy can explain the lower fluorescence values obtained for *B. cereus*, which lead to a lower percentage of cells in the region previously gated as PI-positive. Light microscopy images showed that *B. cereus* cells incubated with an oil concentration of 0.1% (v/v) exhibited a coccus-like shape (Fig. 1e), which was not observed when cells were incubated with DMSO alone (Fig. 1d). This alteration in *B. cereus* morphology has already been described when testing other compounds (Paulo *et al.*, 2010; Si *et al.*, 2006), and the lower nucleic acid content of these cells (Paulo *et al.*, 2010) might explain the lower fluorescence values obtained in our experiments. For an oil concentration of 3.2%, light microscopy images revealed a high presence of cell debris (Fig. 1f), which can also lead to lower fluorescence values and a slight alteration in the FSC and SSC parameters (Fig. 1c).

## Membrane potential

BOX was used to assess membrane potential, since polarized cells are able to exclude anionic molecules such as BOX and depolarized cells allow the accumulation of BOX inside the bacterial cell. The loss of membrane potential is a transition state, because depolarized cells have the ability to regrow on solid media (Díaz *et al.*, 2010).

Overall, the percentage of depolarized cells (BOX-positive) was higher than the percentage of PI-positive cells in oil-treated cells (Tables 2 and 3) owing to the fact that the loss of membrane potential is an event prior to membrane permeabilization (Díaz *et al.*, 2010). The percentage of BOX-positive cells was slightly higher in Gram-positive cells than in Gram-negative cells. Gram-negative bacteria exhibited high fluorescence values of up to 99% and the oil concentration seemed to affect the proportion of BOX-stained cells, as lower oil concentrations tested showed a lower percentage of BOX-stained cells. A high percentage of BOX-stained cells was also obtained for Gram-positive bacteria, excluding *B. cereus* (Table 2), due to the lower fluorescence values obtained caused by the morphological alterations described previously.

### Efflux activity

Due to the importance of efflux pumps as an antibiotic resistance mechanism (Markham & Neyfakh, 2001), it is relevant to study efflux activity when analysing cell physiology. Efflux activity was measured using EB. While the MRSA strains and *E. faecalis* exhibited a lower percentage of stained cells (Table 2), almost 100% of *B. cereus*, *S. aureus* SA08 and *S. aureus* ATCC 25923 cells were EB-positive in the control cultures. This could be due to the fact that these strains do not exhibit efflux pumps for EB exclusion or that the EB concentration used favoured its accumulation inside the cell (Paixão *et al.*, 2009). Meticillin-resistant *S. aureus* strains MRSA 10/08 and 12/08 had a significantly lower percentage of EB-positive cells (26.59 and 34.36%, respectively) when compared to non-resistant strains (99.01 and 96.21%), which could be explained by the higher expression of efflux systems in resistant strains (Poole, 2000). Regarding oil-treated *E. faecalis*, the EB-stained population was higher than in the control culture, which is in accordance with the results obtained for the previous staining protocols.

For oil-treated Gram-negative bacteria, the percentage of EB-stained cells varied between 73.54% and 99.56%. With the exception of *A. baumannii* strains, EB staining of cells appeared to be concentration-dependent, since the highest oil concentrations corresponded to the highest percentages of stained cells (Table 3). For oil-treated *B. cereus*, the percentage of EB-stained cells decreased when compared to the control culture. This can be seen as a result of the lower fluorescence values obtained for these cells, as a result of the lower nucleic acid content exhibited by *B. cereus* coccus-like morphology (Paulo *et al.*, 2010) and cellular debris, which, in turn, lead to a lower percentage of cells in the EB-positive region.

### Respiratory activity

Bacterial cells with respiratory activity are able to reduce CTC, resulting in the production of an insoluble fluorescent CTC–formazan product that can be quantified at low concentrations in individual cells. Control cultures with 2% DMSO contained a high percentage of CTC-reducing bacteria (94.83–99.09%). After incubation with coriander oil, a marked decrease in the ability of bacterial cells to reduce CTC was obtained, even with oil concentrations corresponding to the MBC value. This is demonstrated by a decrease in the percentage of CTC-reducing cells for up to 0–7.5%. There was no significant change in CTC-reducing cells with higher oil concentrations (Tables 2 and 3). This could be explained by the fact that the loss of respiratory activity is one of the first events to take place in the loss of cellular viability (Díaz *et al.*, 2010).

Overall, the correlation between loss of membrane potential, membrane permeability, efflux pump activity, the ability to reduce CTC and reduction of cell viability indicates that coriander oil kills both Gram-positive and Gram-negative bacteria by disrupting membrane function, and so all its functions are compromised not only as a barrier but also as a matrix for enzymes and as an energy transducer. Similar conclusions were reached by other authors attempting to explain the antibacterial action of essential oils using flow cytometry (Hayouni *et al.*, 2008; Nguefack *et al.*, 2004).

Considering that the mode of action of coriander oil against Gram-positive and Gram-negative bacteria seems to be related to membrane permeabilization and that different susceptibility profiles were obtained between Gram-positive and Gram-negative bacteria, it was suggested that coriander oil could have a differential action in the cell wall of both bacteria. In fact, there are some factors that, taken together, might explain the increased susceptibility obtained for Gram-negative bacteria, for instance, the difference in the Gram-negative and Gram-positive bacterial envelope and secondly the composition of coriander oil. In Gram-negative bacteria, the outer membrane has an asymmetrical lipid distribution, with the phospholipids in the inner layer and the lipopolysaccharides, which confer a strong negative charge to the outer membrane, in the outer layer (Trombetta *et al.*, 2005). However, Gram-positive bacteria have a large cell wall formed by peptidoglycan, conferring rigidity (Wu *et al.*, 2008). In coriander oil, linalool, its major component, is found mainly in the form of its *S* (+) enantiomer (Casabianca *et al.*, 1998), which is known to cause increased permeability only in negatively charged membranes (Trombetta *et al.*, 2005). Moreover, alcohols have been related to a decrease of Gram-positive bacterial cell wall thickness, causing an increased resistance of bacteria to compounds such as solvents and dyes (Linhová *et al.*, 2010). In conclusion, the *S* enantiomer of linalool could cause the disruption of the negatively charged Gram-negative bacterial outer membrane whereas its properties as an alcohol could lead to an increased resistance in Gram-positive bacteria due to bacterial cell wall thickening as described above.

These results could lead to a promising use of this essential oil in therapeutic or food applications. The application of this essential oil may be further enhanced by the formulation of delivery systems capable of improving its performance. Despite the fact that some of these formulations have shown a poor release of the essential oil encapsulated (Lai *et al.*, 2007a), other carrier systems based on chitosan or solid lipid nanoparticles enable an effective release of the essential oil from the carrier system with antimicrobial activities similar to or even higher than that of the essential oil alone (Lai *et al.*, 2007b; São Pedro *et al.*, 2009).

Our results demonstrate that, in general, the Gram-positive bacteria tested exhibited lower susceptibility than the Gram-negative bacteria to coriander oil. The flow cytometric assessment of some cellular functions demonstrated that the mode of action of coriander oil is similar in both Gram-positive and -negative bacteria. These flow cytometric experiments enable us to conclude that its primary mode of action seems to be bacterial cell permeabilization. As a result of membrane permeabilization, all other cellular functions such as membrane potential, respiratory activity or efflux pump activity are also compromised. These results, showing a potent antibacterial activity against Gram-positive and Gram-negative bacteria due to membrane permeability, are noteworthy and justify the use of this plant, not only as a food flavouring agent, but also as a food preservative in order to prevent bacterial spoilage of foods. However, this research needs further enlightenment in order to evaluate the suitability of these remarkable antibacterial properties in practical applications.

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**Fig. 1.** Effect of coriander oil on light scatter measurements by flow cytometry and morphology of cells visualized by light microscopy using endospore staining in *B. cereus* ATCC 11778. (a) Light scatter measurements of control cultures with 2% DMSO; (b) light scatter measurements of cells incubated with an oil concentration of 1× MIC value; (c) light scatter measurements of cells incubated with an oil concentration of 3.2% (v/v); (d) light microscopy image of control cells with 2% DMSO; (e) light microscopy image of cells incubated with an oil concentration of 1× MIC value; and (f) light microscopy image of cells incubated with an oil concentration of 3.2% (v/v). A total of 10000 events were collected for this analysis. The numbers on the axes in parts (a)–(c) represent the absolute values on a logarithmic scale.

**Table 1.** Antimicrobial activity (MIC and MBC) of coriander (*Coriandrum sativum* L.) essential oil against Gram-positive and Gram-negative bacterial strains

Each experiment was repeated at least three times at each test concentration and the modal MIC and MBC values were selected.

| Micro-organism | Gram type | MIC (%, v/v) | MBC (%, v/v) |
|---|---|---|---|
| *Bacillus cereus* ATCC 11778 | + | 0.1 | >3.2 |
| *Enterococcus faecalis* ATCC 29212 | + | 0.8 | >3.2 |
| *Staphylococcus aureus* SA08 | + | 0.4 | 3.2 |

| | | | |
|---|---|---|---|
| *Staphylococcus aureus* ATCC 25923 | + | 0.2 | 1.6 |
| MRSA 10/08 | + | 0.2 | 1.6 |
| MRSA 12/08 | + | 0.8 | 3.2 |
| *Pseudomonas aeruginosa* ATCC 27853 | – | 1.6 | 1.6 |
| *Klebsiella pneumoniae* ATCC 13883 | – | 0.2 | 0.2 |
| *Escherichia coli* ATCC 25922 | – | 0.2 | 0.2 |
| *Salmonella typhimurium* ATCC 13311 | – | 0.4 | 0.8 |
| *Acinetobacter baumannii* 2/10 | – | 0.1 | 0.1 |
| *Acinetobacter baumannii* 3/10 | – | 0.2 | 0.2 |

**Table 2.** Percentage of fluorochrome-stained cells evaluated by flow cytometry and viability reduction evaluated by means of the drop-plating method of Gram-positive bacteria treated with coriander oil

The results are presented as mean±standard deviation of two independent samples.

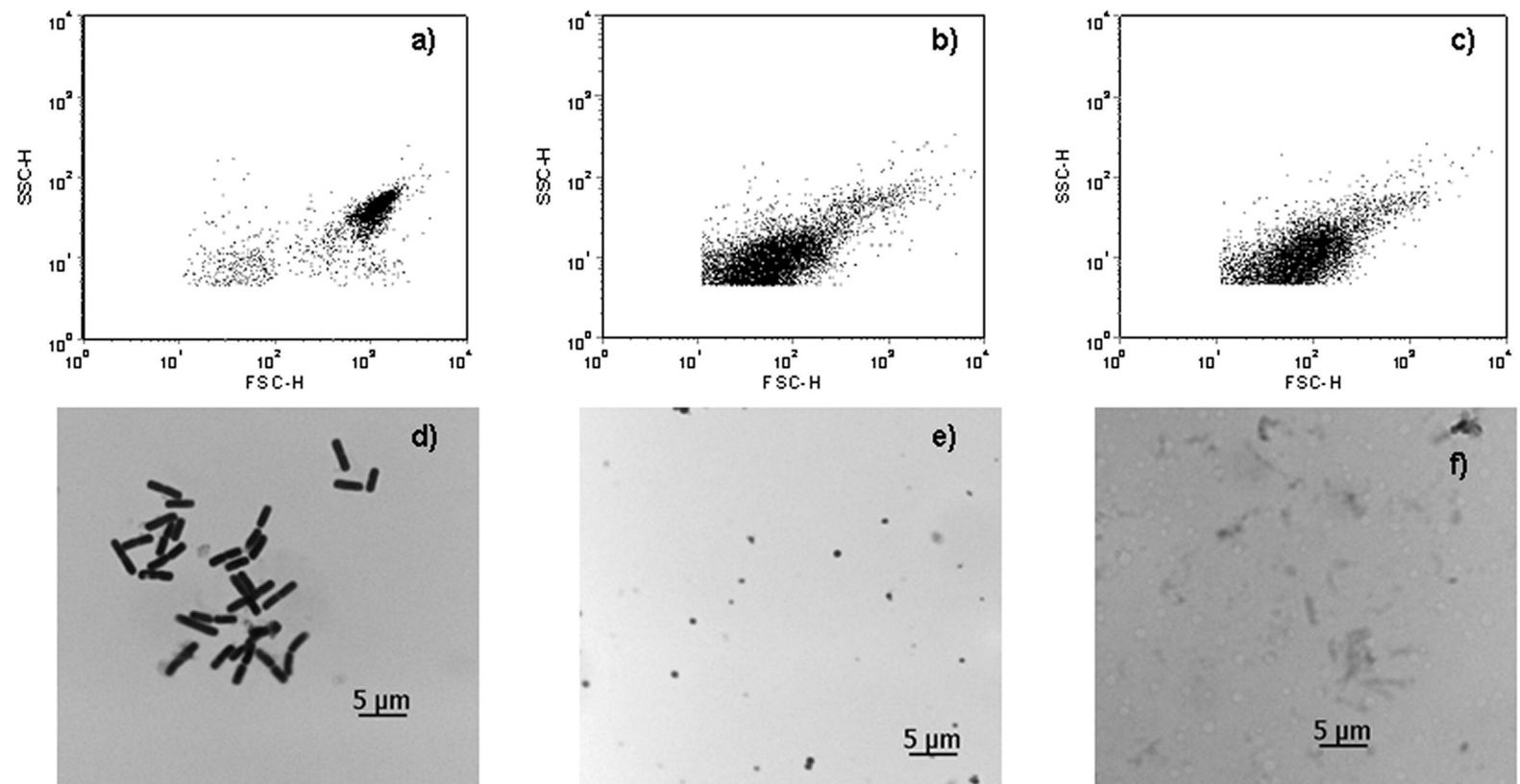
| Micro-organism | Oil concn (%, v/v) | Viability reduction (%) | Stained cells (%) | | | |
|---|---|---|---|---|---|---|
| | | | PI | BOX | EB | CTC |
| *Bacillus cereus* ATCC 11778 | Control (2% DMSO) | 5.50±0.32 | 11.93±1.34 | 2.47±0.44 | 98.39±6.23 | 97.33±5.60 |
| | 0.1 | 99.86±3.41 | 35.62±5.64 | 77.14±3.45 | 40.2±2.21 | 0.42±0.11 |
| | 3.2 | 99.84±3.02 | 55.5±4.34 | 87.67±4.21 | 76.62±3.09 | 7.15±0.12 |
| *Enterococcus faecalis* ATCC 29212 | Control (2% DMSO) | 8.11±1.02 | 14.02±1.21 | 10.59±1.24 | 12.68±1.30 | 96.72±4.56 |
| | 0.8 | 99.17±2.56 | 98.26±3.65 | 98.37±5.32 | 97.32±4.38 | 0.18±0.05 |
| | 3.2 | 99.82±3.42 | 98.95±2.78 | 99.78±5.67 | 95.66±5.91 | 0.16±0.02 |
| *Staphylococcus aureus* SA08 | Control (2% DMSO) | 8.91±0.91 | 15.6±2.73 | 1.65±0.09 | 99.01±6.73 | 96.3±5.67 |
| | 0.4 | 90±2.45 | 88.64±5.87 | 92.16±4.89 | 99.6±4.78 | 0.74±0.21 |
| | 3.2 | 98.73±1.78 | 98.01±3.50 | 99±4.38 | 99.37±5.65 | 0.33±0.09 |
| *Staphylococcus aureus* ATCC 25923 | Control (2% DMSO) | 6.33±1.21 | 13.84±0.34 | 20.73±1.87 | 56.21±2.78 | 95.59±7.21 |
| | 0.2 | 100±0.00 | 94.74±3.75 | 97.69±3.51 | 60.32±2.03 | 5.13±1.34 |
| | 1.6 | 100±0.00 | 98.07±4.67 | 99.26±4.90 | 72.02±1.98 | 4.18±1.55 |
| MRSA 10/08 | Control (2% DMSO) | 9.08±1.67 | 17.33±1.04 | 21.46±1.23 | 26.59±0.98 | 96.9±6.44 |
| | 0.2 | 99.99±0.23 | 91.11±2.23 | 96.49±6.21 | 63.18±2.34 | 0.18±0.04 |
| | 1.6 | 100±0.00 | 98.41±3.43 | 99.55±4.35 | 79.13±1.54 | 0.39±0.03 |
| MRSA 12/08 | Control (2% DMSO) | 6.43±0.58 | 13.11±0.98 | 23.91±2.12 | 34.36±2.13 | 97.83±8.21 |
| | 0.8 | 99.94±2.91 | 91.16±5.02 | 97.26±3.75 | 73.23±3.63 | 0.23±0.06 |
| | 3.2 | 99.99±1.67 | 97.79±4.34 | 99.71±4.02 | 72.75±5.90 | 0.01±0.00 |

**Table 3.** Percentage of fluorochrome-stained cells evaluated by flow cytometry and viability reduction evaluated by means of the drop-plating method of Gram-negative bacteria treated with coriander oil

The results are presented as mean±standard deviation of two independent samples.

| Micro-organism | Oil concn (%, v/v) | Viability reduction (%) | Stained cells (%) | | | |
|---|---|---|---|---|---|---|
| | | | PI | BOX | EB | CTC |
| *Pseudomonas aeruginosa* ATCC 27853 | Control (2% DMSO) | 3±0.34 | 3.54±0.22 | 16.84±2.03 | 4.6±0.62 | 94.83±3.98 |
| | 1.6 | 92±2.45 | 77.62±3.89 | 93.55±0.78 | 73.93±3.47 | 4.69±1.90 |
| | 3.2 | 99.95±0.25 | 86.3±4.05 | 98.06±1.89 | 93.12±5.97 | 5.77±2.01 |
| *Klebsiella pneumoniae* ATCC 13883 | Control (2% DMSO) | 2.56±0.82 | 0.65±0.06 | 2.33±0.07 | 1.42±0.03 | 96.01±5.09 |
| | 0.2 | 100±0.00 | 72.18±0.87 | 93.88±6.72 | 73.54±3.04 | 1.7±0.06 |
| | 0.4 | 100±0.00 | 99.03±3.60 | 99.8±5.21 | 99.56±4.16 | 0.45±0.02 |
| *Escherichia coli* ATCC 25922 | Control (2% DMSO) | 4.17±0.71 | 4.08±0.64 | 8.59±2.90 | 7.52±1.06 | 96.81±0.37 |
| | 0.2 | 100±0.00 | 93.73±2.87 | 94.75±2.76 | 92.89±1.79 | 4.71±0.87 |
| | 0.4 | 100±0.00 | 99.79±4.21 | 99.02±4.41 | 93.18±2.34 | 1.54±0.23 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Salmonella typhimurium* ATCC 13311 | Control (2% DMSO) | 1.88±0.12 | 9.9±2.21 | 13.11±1.20 | 6.99±1.56 | 99.09±6.02 |
| | 0.4 | 100±0.00 | 74.63±2.59 | 95.89±0.87 | 78.63±2.07 | 0.61±0.03 |
| | 0.8 | 100±0.00 | 82.56±3.48 | 96.37±2.90 | 87.3±3.90 | 0.83±0.02 |
| *Acinetobacter baumannii* 02/10 | Control (2% DMSO) | 0.8±0.10 | 4.63±1.12 | 16.34±3.53 | 5.93±0.12 | 98.24±3.45 |
| | 0.1 | 100±0.0 | 96.19±2.18 | 97.10±5.35 | 97.28±1.89 | 0.58±0.02 |
| | 0.2 | 100±0.0 | 96.72±3.67 | 97.72±4.65 | 97.47±3.21 | 0.65±0.01 |
| *Acinetobacter baumannii* 03/10 | Control (2% DMSO) | 1.67±0.12 | 0.95±0.02 | 13.27±2.31 | 3.02±0.54 | 99.02±5.67 |
| | 0.2 | 100±0.0 | 98.09±4.32 | 99.03±2.31 | 98.70±3.87 | 2.65±0.30 |
| | 0.4 | 100±0.0 | 98.73±3.29 | 99.02±1.56 | 98.64±2.12 | 1.56±0.40 |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