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RESEARCH ARTICLE

Anti-quorum sensing and antimicrobial activity of aromatic species from South America

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Quorum sensing (QS) is a bacterial communication mechanism that depends on population density. The interruption of QS is one example of an antipathogenic effect. We investigated the anti-QS and antimicrobial properties of essential oils from Argentina: *Salvia officinalis*, *Minthostachys mollis*, *Satureja odora*, *Schinus molle*, *Lepechinia floribunda* and *Artemisia annua.* Anti-QS activity was determined by measuring the production of violacein in *Chromobacterium violaceum* through UV–visible spectrophotometry and the minimal QS inhibitory concentration (MQSIC) was calculated. The antimicrobial activity was determined using *Escherichia coli*, *Listeria innocua* and *Staphylococcus aureus* as indicators. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by performing the broth microdilution assay. *Minthostachys mollis* showed statistically significant QS inhibition properties. This essential oil reduced pigment production by 90% when it was applied at a sublethal concentration (0.02% v/v). Conversely, the highest bacteriostatic and bactericidal activity was exhibited by *S. molle* oil. *Minthostachys mollis* essential oil is a good candidate for the development of anti-QS products with a potential application in the control of bacterial diseases mediated by QS. As this strategy interferes with the expression of pathogenic traits rather than killing the microorganism or impeding microbial growth, it avoids the problem of resistance.

Keywords: *Chromobacterium violaceum*; *Escherichia coli*; *Listeria innocua*; *Staphylococcus aureus*; essential oils; quorum sensing

1. Introduction

Essential oils are natural, volatile and complex products obtained as secondary metabolites from aromatic plants and have different biological effects. They may act as anticancer, anti-inflammatory, insect repellent, antimicrobial and antiviral substances, as well as antioxidant agents. As essential oils affect many targets at the same time, no cases of resistance or adaptation have been detected, as occurs with the use of antibiotics (1).

In Argentina, 602 plant species are known to possess therapeutic properties. Many research studies in Córdoba province have shown that the essential oils obtained from *Lippia turbinata*, among others, act as antimicrobial agents against different microorganisms. Moreover, extracts from *Minthostachys mollis* were found to have inhibitory effects on certain viruses (2). Palacios et al. (3) studied the insecticidal activity found in *Minthostachys verticillata* and *Artemisia annua* essential oils from Córdoba on the domestic fly. *Lippia turbinata* and *Satureja parvifolia* collected from Tucumán presented antimicrobial activity against different Gram-positive and Gram-negative microorganisms (4).

Plants produce several antimicrobial compounds such as phenols, alkaloids, terpenes, flavonoids, etc., which have the cell membrane of various microorganisms as their action target. However, it is currently known that essential oils can act in different ways when administered in sublethal concentrations, for instance blocking or interrupting bacteria communication mechanisms (5), known as quorum sensing (QS). QS involves molecules called autoinducers, which activate receptors that allow the transcription of a battery of genes. The expression of these genes controls different biochemical mechanisms involved in bacterial survival and pathogenicity (virulence). In this way, QS regulates a diversity of bacterial functions like luminescence, biofilm formation, production of antibiotics, virulence factors and pigments, plant–microorganism interaction and motility (6). As QS regulated the mechanism of microbial infection, the inhibition of cell-to-cell communication can lead to decrease the infection (7).

In contrast to antimicrobial compounds, anti-quorum sensing (anti-QS) or 'anti-pathogenic' compounds

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do not cause cell death or growth arrest. Some studies have demonstrated the potential use of different plant oils with anti-QS activity (for instance clove, cinnamon, peppermint, lavender, rosemary, rose and geranium) (8–10). *Chromobacterium violaceum* is a Gram-negative water and soil bacterium that produces violacein, a water-insoluble purple pigment, as a phenotypic response regulated by a QS mechanism. Therefore, this bacterium is used as a bioindicator to detect substances that block the QS mechanism (11).

Previous anti-infective studies on medicinal plants concentrated mainly on the validation of antimicrobial potential for traditional use. Several biological properties of the essential oils from *Salvia officinalis*, *M. mollis*, *Satureja odora*, *Schinus molle*, *Lepechinia floribunda* and *A. annua*, such as antimicrobial, antifungal, insecticidal, antiviral, anticarcinogenic, antioxidant and antiparasitic, have already been demonstrated (3, 12–17). However, as far as we are concerned, these agents have never been reported as QS inhibitors.

The purpose of this study was to investigate the anti-QS and antimicrobial properties of the essential oils of different plants from Argentina: *S. officinalis*, *M. mollis*, *S. odora*, *S. molle*, *L. floribunda* and *A. annua*. Their anti-QS activity was evaluated in order to determine the minimal concentration of essential oils that, by interrupting QS, would inhibit bacterial pathogenicity but not its growth. QS inhibitory activity of essential oils was determined by measuring the production of violacein in *C. violaceum*. Moreover, *Escherichia coli*, *Listeria innocua* and *Staphylococcus aureus* were used to determine the antimicrobial activity of the studied essential oils.

2. Experimental

2.1 Bioactive substances

The essential oils of *S. officinalis* from Mar del Plata (Argentina), *A. annua*, *L. floribunda*, *S. molle*, *S. odora* from San Luis (Argentina), and *M. mollis* from Córdoba (Argentina) were obtained through steam distillation. The chemical compositions of these essential oils were determined in previous reports by Fuselli et al. (18, 19) and Fuselli (20) by solid-phase microextraction (SPME) coupled to gas chromatography–mass spectrometry (GC/MS) analysis (Table 1). GC/MS analyses were carried out on an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) coupled to an Agilent 5970 mass selective detector operating in electron impact mode (ionization voltage, 70 eV). A Chrompack CP-Wax 52 CB capillary column (50 m length, 0.32 mm, 1.2 μm df) was used (Chrompack, Middelburg, The Netherlands). The temperature program was as follows: initial temperature at 50°C for 1 minute, then the temperature was increased to 65°C at 1°C/minute and then to 220°C at 5°C/minute. The injector and detector were at 250°C. Injections were performed with a split ratio of 1:20 and He was used as the carrier gas at a flow rate of (1 mL/minute). A polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (1 cm length and 65 μm width; Supelco Inc., Bellefonte, PA) was used for SPME. Two milliliters of the oil sample were placed in 2-mL vials sealed with PTFE/silicone septa. The samples were equilibrated for 15 minutes at 50°C. The SPME fiber was exposed to each sample for 5 minutes by manually penetrating the septum. Then, the fiber was inserted into the injection port of the GC for sample desorption for 5 minutes. Before each headspace sampling, the fiber was exposed to the GC inlet for 5 minutes for thermal desorption at 250°C on a blank run. The retention index was calculated using a homologous series of *n*-alkanes (C_8–C_{28}) in the same conditions as detailed above. Compounds were identified based on their relative retention indices, and by comparison of the mass spectra of the essential oil components to the mass spectra of standards and the mass spectra recorded in the data library of the National Institute of Standards and Technology–United States Environmental Protection Agency–National Institute of Health (21) and Wiley (22). Quantitative data is reported as a percentage of each peak area related to the total area of the peaks determined.

2.2 Strains and culture conditions

Chromobacterium violaceum wild-type strain ATCC (American Type Culture Collection) 12472 (Malbrán Institute, Argentina) was used for the anti-QS assays. This strain was grown under aerobic conditions in Luria–Bertani broth (LB) incubated at 30°C for 24 hours.

Moreover, *E. coli* O157:H7 ATCC 25158, *L. innocua* CIP 8011 and *S. aureus* ATCC 25923 were used for the antimicrobial assays. The strains were pre-cultured in brain–heart infusion (BHI, Britania) for 24 hours at 37°C. Each culture (0.1 mL) was transferred to 9.9 mL of BHI at two consecutive 24-hour intervals immediately before each experiment.

2.3 Anti-QS activity assays: disk diffusion method

In order to obtain a qualitative screening of the essential oils inhibition activity, a disk diffusion method was used with *C. violaceum* as a biosensor strain. This bacterium produces a purple pigment (violacein) as a response to autoinducer synthesis (11). A 0.1-mL aliquot of a fresh *C. violaceum* culture dilution (2.5×10^6 CFU/mL) was plated on LB agar Petri dishes (LB broth supplemented with 1.5% bacteriological grade

Table 1. Composition of essential oils.

Compounds[a]	RI[b]	Essential oils					
		Salvia officinalis	*Artemisia annua*	*Lepechinia floribunda*	*Schinus molle*	*Satureja odora*	*Minthostachys mollis*
		Area (%)					
α-Pinene	1034	13.4	–	10.9	4.1	1.7	–
Camphene	1115	8.8	–	16.6	7.9	–	–
β-Pinene	1117	1.6	–	5.4	3.2	2.5	–
β-Myrcene	1143	1.8	8.3	–	5.3	1.7	–
α-Terpinene	1158	–	3.1	–	–	–	–
Limonene	1186	2.8	–	6.1	–	15.1	2.6
α-Phellandrene	1205	–	–	–	11.5	–	–
1,8-Cineole	1210	9.1	31.5	27.5	–	–	–
β-Phellandrene	1241	–	–	–	34.3	–	–
cis-β-Ocimene	1245	–	–	1.3	–	–	–
γ-Terpinene	1263	0.9	–	0.9	–	–	–
p-Cymene	1280	1.5	–	–	–	–	–
β-Thujone	1404	11.9	–	–	–	–	–
α-Thujone	1419	25.2	–	–	–	–	–
Caryophyllene	1420	–	–	6.3	2.6	3.4	7.2
Isomenthone	1429	–	–	–	–	1.1	1.5
D-Menthene	1455	–	–	–	–	–	35.8
Menthone	1474	–	–	–	–	32.5	–
Camphor	1501	19.4	20.6	12.9	–	–	–
Ketone artemisia	1509	–	36.3	–	–	–	–
Elemene	1580	–	–	–	5.6	–	–
Pulegone	1601	–	–	–	–	42	52.6
Borneol	1642	–	–	5.6	–	–	–
Muurolol	1644	–	–	–	3.6	–	–
D-Germacrene	1715	–	–	–	2.1	–	–
Bicyclogermacrene	1738	–	–	–	1.6	–	–
Caryophyllene oxide	1999	–	–	–	7.9	–	–

Notes: [a]Compounds are listed in their elution order on a CP-Wax 52 CB column. [b]RI, retention indices on column CP- Wax 52 CB with a stationary phase of polyethylene glycol determined using a homologous series of *n*-alkanes (C_8–C_{28}).

agar) (23). Sterile paper disks were impregnated with pure essential oil and placed above the agar. A negative control was performed by impregnating the paper disk with sterile LB broth. The Petri dishes were incubated at 30°C for 18–24 hours. Pigment production inhibition was determined by measuring the growth inhibition diameters around the disks. The susceptibility of *C. violaceum* against the tested oils was classified according to the halo diameter as follows: 'not sensitive' for diameters less than 8 mm, 'sensitive' for diameters between 9 and 14 mm, 'very sensitive' for diameters between 15 and 19 mm, and 'extremely sensitive' for diameters greater than 20 mm (24).

2.4 Anti-QS activity assay: macrodilution method

In order to quantify the anti-QS activity of the essential oils, *C. violaceum* (1×10^8 CFU/mL) was incubated in the presence of different oil concentrations. *Chromobacterium violaceum* was inoculated in Erlenmeyer flasks containing LB supplemented with essential oils to obtain different concentrations (0.005, 0.01, 0.02, 0.04, 0.1 and 0.2% (v/v) for *S. officinalis*; 0.005, 0.01, 0.02, 0.04, 0.06, 0.1, 0.2 and 0.3% (v/v) for *A. annua;* 0.01, 0.02, 0.04, 0.06, 0.1, 0.2 and 0.3% (v/v) for *L. floribunda*; 0.0025, 0.005, 0.01, 0.02, 0.04, 0.06 and 0.1% (v/v) for *S. molle* and 0.0025, 0.005, 0.01, 0.02, 0.04, 0.1 and 0.2% (v/v) for *S. odora* and *M. mollis*). The flasks were incubated at 30°C in a shaking incubator for 24 hours. Violacein production was quantified following Choo et al.'s (25) protocol: 1 mL culture of each test tube was centrifuged at 13,000 rpm for 10 minutes to precipitate the insoluble pigment. The pellet was resuspended in 1 mL of dimethyl sulfoxide (DMSO; Biopack, Argentina) and homogenized by vortexing. Violacein absorbance at 585 nm was determined using a UV–visible spectrophotometer (Shimadzu Corp., Kyoto, Japan). The control sample consisted of incubating the microorganism in LB broth without adding the essential oils.

The inhibition or decrease in violacein production can be a direct result of: (i) blockage of QS mechanisms or (ii) inhibition of cell growth (26). The antimicrobial activity of essential oils against

C. violaceum was evaluated. To determine the bacterial concentration, dilutions with sterile peptone water were performed in each test tube solution, which were then plated on LB agar dishes and incubated at 30°C for 24 hours. Colonies were counted and expressed as log CFU/mL.

2.5 Antimicrobial activity assays: microdilution method

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values were determined on *E. coli*, *L. innocua* and *S. aureus* strains using the microdilution method. Essential oil stock solution was prepared by diluting each essential oil with brain–heart broth and emulsifying with Tween-80 0.05% (v/v). In a 96-multiwell plate, each well was filled with 100 µL of brain–heart broth except for the first column. Furthermore, 200 µL of each essential oil stock solution was placed in the first column and a serial dilution was performed up until to the eleventh column. An aliquot of 5 µL of the bacterial culture (to obtain a final inoculum concentration of 5×10^5 CFU/mL) was placed inside each well. The twelfth column held the viability control (no oils were added). The multiwell plates were incubated at 30°C for 24 hours under normal atmospheric conditions. Analyses were performed in triplicate. The bacterial growth was indicated by the presence of a white pellet on the bottom of the well. The MIC is defined as the lowest concentration of the oil that is able to inhibit visible growth of the microorganism (27). To establish MBC, 100 µL of the contents of each well without a pellet was removed, transferred to LB agar Petri dishes and incubated at 30°C for 24 hours; CFU/mL was determined on each plate. MBC is considered the lowest concentration capable of killing 99.9% of the initial inoculum (27).

2.6 Statistical analysis

The mean values and the standard deviation were calculated from the data obtained from triplicate trials. To analyze the violacein production data, a Probit analysis was performed with the Toxstat 3.0 software (Gulley, Boelter and Bergman; University of Wyoming, USA) to determine the concentration of essential oil that decreased pigment production by 50% with 95% confidence. Analysis of variance (ANOVA) was applied to the data in each experiment to determine the statistical differences between treatments. Furthermore, the data was compared using Tukey's test for significant effects. For all cases, the level of significance was set at $p < 0.05$. Statistical analysis was developed using R 2.12.2 statistical software (2010).

3. Results and discussion

3.1 Anti-QS activity of essential oils

In this study, essential oils from *S. officinalis*, *A. annua*, *S. odora*, *S. molle*, *L. floribunda* and *M. mollis* were evaluated as anti-QS substances for the first time.

In order to obtain a qualitative screening of the QS inhibitory activity of essential oils, the disk diffusion method was used with *C. violaceum* as an indicator strain (Table 2). The loss of purple pigment in *C. violaceum* and the presences of turbid halos are indicatives of QS inhibition by the essential oils without affecting microbial growth. Clear halos represent antibacterial activity. According to the measurement of the inhibition halos, the production of violacein was 'extremely sensitive' for all oils except for *L. floribunda* that turned out to be 'sensitive'. Besides anti-QS activity, antibacterial activity was also observed in all tested essential oils (Table 2). All essential oils studied except for *L. floribunda* showed QS inhibition halos bigger than those measured for the antimicrobial activity, demonstrating their potential as QS inhibitory agents. In the case of the essential oils of *M. mollis* and *S. odora*, differences between inhibition halos were even more pronounced.

The essential oil of *L. floribunda* showed the same mean diameter for pigment inhibition and growth inhibition; the reduction in the production of violacein was due to an antimicrobial effect of this oil against *C. violaceum*. According to this methodology, *M. mollis* and *S. odora* were the most effective essential oils in inhibiting the QS mechanism in *C. violaceum*.

The QS inhibitory activity of essential oils was also evaluated by quantifying the production of violacein in *C. violaceum*. In order to determine whether the inhibition in the production of violacein was due to a blockage in cellular communication rather than to inhibition in cell growth, cell viability was monitored after treatments. Figure 1 shows the percentage of violacein production and *C. violaceum* counts (expressed as log CFU/mL) as a function of essential oil concentration. In general, pigment production and *C. violaceum* counts showed an inverse relationship with essential oil concentrations. Depending on the concentrations tested, the oils showed different levels of effectiveness as QS inhibitors. *Satureja odora*, at the concentrations tested, exerted no significant QS inhibitory activity ($p > 0.05$) because a reduction in the production of violacein was observed along with cell death. *Schinus molle*, *S. officinalis* and *L. floribunda* oils caused a 50% decrease in the production of violacein when used at 0.005% (v/v), 0.04% (v/v) and 0.06% (v/v), respectively. The essential oil of *A. annua* applied at 0.1% (v/v) reduced pigment production by 80%. However, at 0.02% (v/v), only *M. mollis* essential oil was able to significantly inhibit violacein production (90%

Table 2. Diameter of quorum sensing (QS) inhibition and antimicrobial halos for each pure essential oil.

Essential oils	Halos	
	Diameter of QS inhibition halos (mm ± SD)	Diameter of antimicrobial halos (mm ± SD)
Salvia officinalis	>90 ± 0.1	12 ± 0.3
Artemisia annua	70 ± 0.2	10 ± 0.1
Lepechinia floribunda	14 ± 0.1	14 ± 0.2
Schinus molle	50 ± 1.2	24 ± 0.8
Satureja odora	90 ± 0.1	6 ± 0.1
Minthostachys mollis	>90 ± 0.1	6 ± 0.1

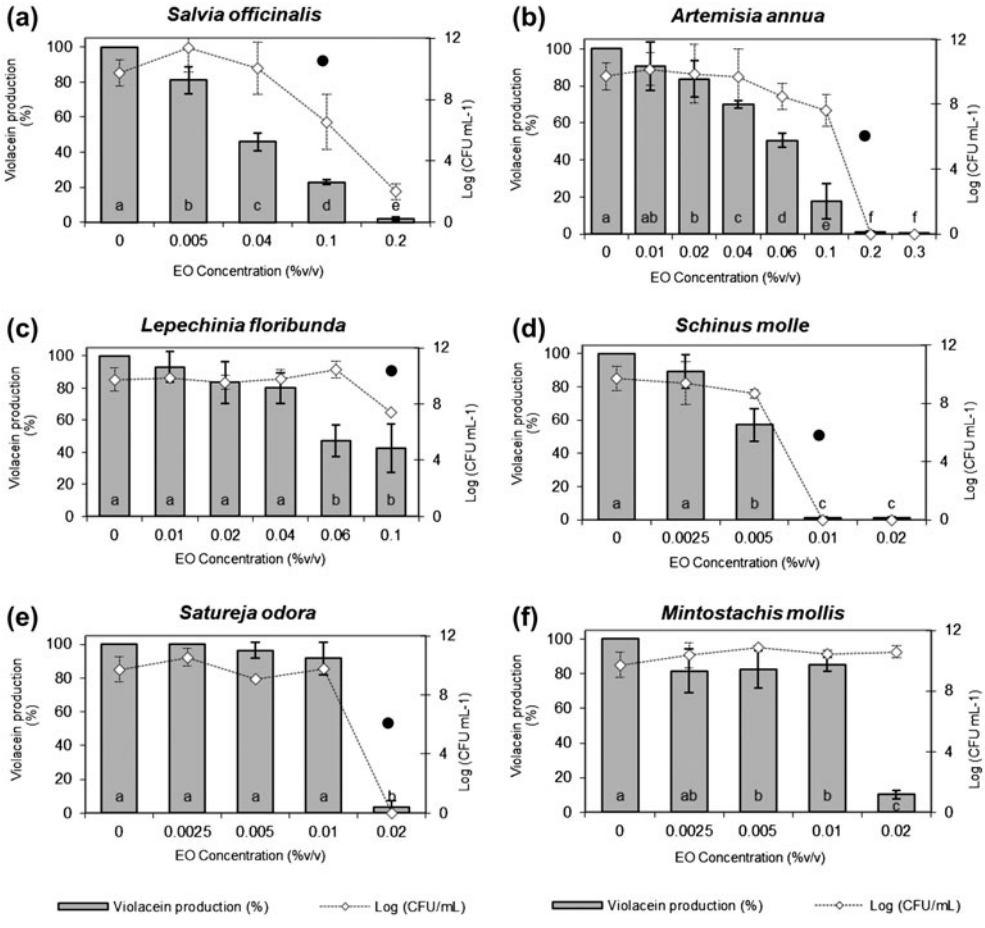

Figure 1. Effect of increasing concentrations of essential oils on growth and violacein production by *Chromobacterium violaceum*. Absorbance data was transformed into percentages with the untreated (control) set as 100%. Vertical bars and empty diamonds represent means of three replicates ± standard deviation. Mean values of violacein production (left axis) with different letters are significantly different at $p < 0.05$. Mean values of log (CFU/mL) (right axis) with (•) are significantly different at $p < 0.05$.

of reduction; $p < 0.05$), whereas bacterial counts remained at the same level when compared with the control sample ($p > 0.05$). Therefore, such a decrease in the violacein production was proved to be due to a blockage in the QS mechanism without affecting bacterial growth.

Taking into account these results, the minimum QS inhibitory concentration (MQSIC) of the different bioactive oils was estimated (Table 3). The MQSIC is defined as the effective concentration of bioactive agent at which 50% of the QS activity was reduced (28). MQSIC values ranged between 0.005% and 0.073%

Table 3. Minimal quorum sensing inhibitory concentrations (MQSICs) of essential oils against *Chromobacterium violaceum* and *C. violaceum* counts corresponding to the application of MQSIC.

	Chromobacterium violaceum			
			Cell viability (log CFU/mL)	
Essential oil	MQSIC (%v/v)	Confidence limits	Control	MQSIC
Salvia officinalis	0.0259	0–0.1058	10.0[a]	9.4[a]
Artemisia annua	0.0649	0.058–0.07	10.0[a]	9.3[a]
Lepechinia floribunda	0.0734	0.1942–0.048	10.0[a]	9.5[a]
Schinus molle	0.005	0.0054–0.0045	10.0[a]	9.8[a]
Satureja odora	0.0138	0.0129–0.0148	10.0[a]	9.5[a]
Minthostachys mollis	0.0137	0.0124–0.0149	10.0[a]	10.1[a]

Note: [a]Means followed by the same letter are in a row are not significantly different at $p < 0.05$.

(v/v). *Schinus molle*, *M. mollis and S. odora* showed the lowest MQSIC values, demonstrating their high anti-QS capacity.

Zaki et al. (28) evaluated the anti-QS activity of an ethanolic extract obtained from the leaves of *S. molle* containing polyphenols, steroids and triterpenoids. This ethanolic extract was not effective in inhibiting violacein production by *C. violaceum.* In contrast, *S. molle* essential oil tested in our study was effective as a QS inhibitor. This indicates that the compounds responsible for the anti-QS activity are contained only in the volatile fraction.

The effect of essential oils, applied at MQSIC, in the growth of *C. violaceum* was verified experimentally through the macrodilution method. None of the oils tested showed antimicrobial activity at these concentrations (Table 3).

Plant essential oils contain a mixture of various active compounds. Thus, it is difficult to comment on the exact mode of action on the QS system. In this regard, Olivero et al. (10) suggested that essential oils might be acting through a possible competitive inhibition with the autoinducer receptor due to the apolar nature and relative size of the components of essential oils (similar to autoinducers in Gram-negative QS system).

3.2 *Antimicrobial activity of essential oils*

To validate some aspects of the traditional uses of the tested agents as antimicrobials, essential oils were tested through a microdilution assay against Gram-positive (*L. innocua* and *S. aureus*) and Gram-negative bacteria (*E. coli*). MIC and MBC values were determined.

In the present study, all the bacterial strains demonstrated some degree of sensitivity against the essential oils tested. *Schinus molle* and *M. mollis* showed higher antimicrobial efficacy than the other oils (lowest MIC and MBC values) against *E. coli* (Table 4). The essential oil of *L. floribunda* showed the highest MIC and MBC values; *E. coli* strain proved the most resistant strain when this oil was tested (Table 4). Regarding Gram-positive bacteria, both strains were found to be more susceptible to *S. molle* essential oil than to the others oils. *S. aureus* was also susceptible to *M. mollis* essential oil. In addition, *A. annua* and *S. odora* oils had the lowest bacteriostatic and bactericidal effect on Gram-positive bacteria. The essential oil of *S. molle* was the most effective antimicrobial agent on *E. coli*, *S. aureus* and *L. innocua*. Our results are in agreement with Guerra-Boone et al. (29), who found that *S. molle* essential oil, from the northeast of Mexico, was effective in inhibiting *S. aureus* growth. Also, Mora et al. (30) reported that *M. mollis* essential oil from Venezuela exerted a high antimicrobial activity against several microorganisms, being more effective against *Bacillus subtilis* and *Salmonella typhi*.

Burt (31) reviewed the antibacterial properties of essential oils; most studies agree that essential oils are slightly more active against Gram-positive than Gram-negative bacteria. Nevertheless, a study testing the antimicrobial action of fifty commercially available essential oils against twenty-five genera found no evidence for a difference in sensitivity between Gram-negative and Gram-positive bacteria (32). In the present study, it was not possible to make a generalization about the susceptibility of bacteria to the essential oils studied according to the type of bacteria. Regarding the results shown in Table 4, *S. officinalis* and *L. floribunda* showed a higher antibacterial effect against Gram-positive bacteria. Conversely, *S. odora* was more effective against the Gram-negative bacterium *E. coli*. *Artemisia annua*, *S. molle* and *M. mollis* showed similar effectiveness against both type of bacteria. Similarly, Dorman and Deans (33) postulated that individual components of essential oils exert different degrees of activity against Gram-positive and Gram-negative microorganisms.

The chemical compositions of essential oils depend on climatic, seasonal and geographical conditions, harvest period and distillation technique. The antimicrobial activities of essential oils depend on the chemical composition, concentration, storage conditions (34) and target microorganism. In the literature, there are examples

Table 4. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of essential oils against *Escherichia coli*, *Listeria innocua* and *Staphylococcus aureus*.

	Organism					
	Escherichia coli		*Listeria innocua*		*Staphylococcus aureus*	
Essential oil	MIC (%v/v)	MBC (%v/v)	MIC (%v/v)	MBC (%v/v)	MIC (%v/v)	MBC (%v/v)
Salvia officinalis	1.28	2.56	0.64	5.12	0.64	5.12
Artemisia annua	1.28	2.56	1.28	>5.12	2.56	>5.12
Lepechinia floribunda	2.56	5.12	0.64	2.56	0.64	5.12
Schinus molle	0.32	0.64	0.32	0.64	0.32	0.64
Satureja odora	0.64	2.56	5.12	>5.12	2.56	>5.12
Minthostachys mollis	0.32	0.64	0.64	2.56	0.32	1.28

where correlation between the major components of the essential oils and the antimicrobial activity has been shown. 1,8-Cineole (the main component of *A. annua* and *L. floribunda*) and camphor (another major component of *A. annua*) are chemicals known to have antimicrobial activity (35). Moreover, using chemometric analysis, camphor was found to be a putative biomarker responsible for the antimicrobial activities of various oils (36). Artemisia ketone, another constituent of *A. annua*, was demonstrated to be responsible for the antimicrobial activity in several Gram- negative and Gram-positive strains (37). In the case of *S. officinalis*, its major component is alpha-thujone, to which the antimicrobial activity of the oil is attributed (38). Pulegone (major component of *S. odora* and *M. mollis* essential oils) is known to have antibacterial properties against several bacteria species (39). In general, the cytotoxic activity of essential oils is mostly due to the presence of phenols, aldehydes and alcohols (40).

In conclusion, in the present study, the results indicated that *M. mollis* essential oil applied at sublethal concentrations acted as the most effective QS inhibitory agent, reducing the pigment production in *C. violaceum* by 90%. For that, *M. mollis* essential oil is a good candidate for the development of anti-QS products with a potential application in the control of bacterial diseases mediated by QS. In addition, *S. officinalis*, *A. annua* and *S. molle* showed a circumscribed anti-QS activity, depending on the concentration of essential oil used. However, *L. floribunda* and *S. odora* essential oils provided different results depending on the type of test used; therefore, it is not possible to conclude consistently on their potential as QS inhibitors. Regarding the antibacterial capacity of the essential oils, *S. molle* oil exhibited the highest bacteriostatic and bactericidal activity against *E. coli*, *S. aureus* and *L. innocua*.

In further research, it will be interesting to investigate whether pathogens utilize QS as part of their pathogenic lifestyle, and if so, whether the production of the signal molecules, i.e. autoinducers, can be diminished by using essential oils.

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