Phytomedicine xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Inhibition of quorum sensing in *Pseudomonas aeruginosa* by sesquiterpene lactones

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ARTICLE INFO

Keywords:
Centratherum punctatum
Biofilm
Quorum sensing
Sesquiterpene lactones
Pseudomonas aeruginosa
Elastase

ABSTRACT

Six sesquiterpene lactones (SLs) of the goyazensolide and isogoyazensolide-type isolated from the Argentine herb *Centratherum punctatum* were evaluated on their ability to inhibit virulence factors of *Pseudomonas aeruginosa* ATCC 27853.

Although compounds were not able to completely inhibit bacterial growth at 200 μg/ml, the SLs do altered biofilm formation, elastase activity, and production of N-acyl-homoserinelactones (AHLs) which are known quorum sensing autoinducers at lower concentration. Compounds **2**, **3**, and **5** displayed significant inhibitory effects on *P. aeruginosa* biofilm formation at 0.5 μg/ml being compound **3** (1.32 μM) the most potent (42%). Compounds **2**, **3**, **4**, **5** and **6**, inhibited 39, 44, 42, 32 and 35% the production of AHLs at 100 μg/ml and inhibited by more than 50% the elastase activity at 0.5 μg/ml. Our results clearly indicated that sesquiterpene lactones are good candidates for the development of new antimicrobial agents acting not as bactericidal but as antipathogenic agents.

Introduction

Quorum sensing (QS) is a term first used to describe an environmental sensing system that allows bacteria to monitor their own population density (Fuqua et al. 1994). After knowing the importance of QS during bacterial pathogenesis, research has focused on inhibiting QS in order to avoid bacterial infections (Adonizio et al. 2006). The interruption of QS, or bacterial cell-to-cell communication, is one example of an antipathogenic effect. Since a large number of systems affecting pathogenicity are controlled by QS, interrupting this communication system can render pathogenic bacteria non-virulent (Fuqua et al. 1994). *Pseudomonas aeruginosa*, as most Gram-negative bacteria, produces N-acyl-homoserinelactones (AHLs) as signal molecules. One of the main consequences of QS in Gram-negative bacteria is biofilm formation. Biofilm-growing bacteria are more resistant to antibiotics than their planktonic counterparts (Zhang and Dong 2004).

Furthermore, *Pseudomonas* elastase, also known as pseudolysin or LasB, is a metalloprotease, which has long been recognized as a key virulence factor produced by *P. aeruginosa* (Stewart and Costerton 2001). The secreted protease degrades a broad range of host tissue proteins and key biomolecules involved in innate immunity such as immunoglobulins, complement factors and cytokines (Liu 1974). In addition, LasB acts within the bacterial cell as a key regulator in the generation of the secreted polysaccharides that constitute the bacterial biofilm (Cathcart et al. 2009), thus LasB inhibition could be important for attenuation of *Pseudomonas* virulence (Sokol et al. 2000).

Antipathogenic compounds do not kill bacteria or stop their growth. They rather control bacterial virulence factors and prevent the development of resistant strains (Otto 2004). The available data indicate that plants might produce a range of AHL-inhibitory compounds (Rice et al. 2005). Anti-QS activity has been shown in a number of southern Florida seaweeds (Cumberbatch 2002) and a few terrestrial plants (Gao et al. 2003; Gilabert et al. 2011; Teplitski et al. 2000). In addition, the influence of few secondary metabolites from South American plants on biofilm formation has been reported by Cartagena et al. (2007), Gilabert et al. (2011), and Socolsky et al. (2010).

Continuing with our investigations on bioactive constituents of plant origin we evaluated and report in the present article the effects of six sesquiterpene lactones (Fig. 1), three of the goyazensolide-type (**2**, **3**, and **5**) and three of the isogoyazensolide-type (**1**, **4**, and **6**) isolated from *Centratherum punctatum* Cass. ssp. *punctatum* (Compositae) on the bacterial

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http://dx.doi.org/10.1016/j.phymed.2012.07.003

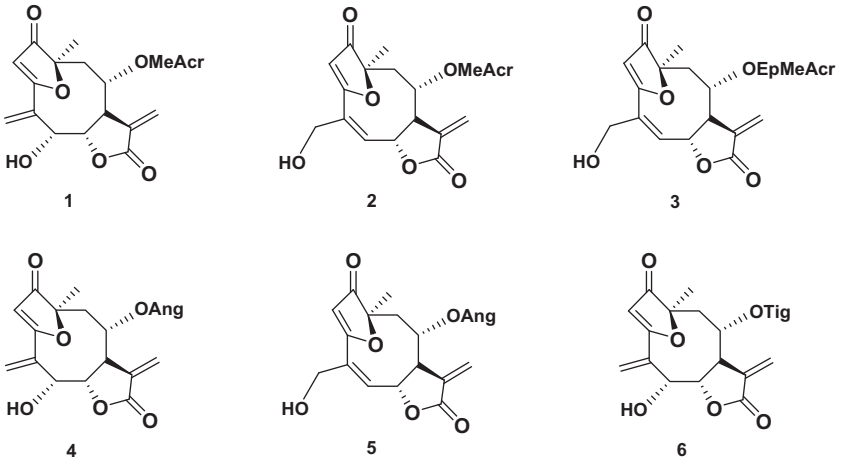

Fig. 1. Sesquiterpene lactones isolated from *Centratherum punctatum* ssp. *punctatum*. IUPAC names of the compounds assayed: Compound **1** – 1-Oxo-3, 10-epoxy-5-hydroxy-8-metacryloyloxy-germacra-2,4(15),11(13)-trien-6,12-olide. Molecular weight: 360. Compound **2** – 1-Oxo-3, 10-epoxy-8-methacryloyloxy-15-hydroxygermacra-2,4,11(13)-trien-6,12-olide. Molecular weight: 360. Compound **3** – 1-Oxo-3,10-epoxy-8-epoxymethacryloyloxy-15-hydroxygermacra-2,4,11(13)-trien-6,12-olide. Molecular weight: 376. Compound **4** – 1-Oxo-3,10-epoxy-5-hydroxy-8-angeloyloxy-germacra-2,4(15),11(13)-trien-6,12-olide; common name: Isocentratherin. Molecular weight: 374. Compound **5** – 1-Oxo-3,10-epoxy-8-angeloyloxy-15-hydroxygermacra-2,4,11(13)-trien-6,12-olide; common name: Centratherin. Molecular weight: 374. Compound **6** – 1-Oxo-3,10-epoxy-5-hydroxy-8-tigloyloxy-germacra-2,4(15),11(13)-trien-6,12-olide. Molecular weight: 374.

growth, biofilm formation, elastase activity, and production of N-acyl-homoserinelactones (AHLs) of *P. aeruginosa*.

Materials and methods

*Extraction, purification and identification of SLs **1**–**6***

Aerial parts of *C. punctatum* ssp. *punctatum* were collected at the flowering stage in Misiones Province, Argentina. A voucher specimen Lil # 611039 is on deposit at the herbarium of Fundación Miguel Lillo, Tucumán, Argentina. The compounds were extracted, purified and unambiguously identified by its IR, NMR and MS spectra by comparison with previously reported data (Álvarez Valdés et al. 1998). Compounds **1**, **4** and **6** were isolated from leaves while compounds **2**, **3** and **5** were isolated from flowers.

Bacterial growth

Overnight cultures of *P. aeruginosa* ATCC 27853 were diluted to reach an OD (0.125 ± 0.02) at 560 nm in Luria–Bertani (LB) medium. The diluted culture (190 μl) was placed in each of the 96 wells of a microtitre polystyrene plate. Solutions containing 20, 10, 1, 0.1 and 0.01 mg/ml of compounds **1**–**6** in DMSO/distilled water (1:1) were prepared separately and 10 μl of each was pipetted to the plastic microtitre plate wells individually (8 replicates). Control wells (8 replicates) contained the diluted culture (190 μl) and 10 μl of a solution of DMSO/water (1:1) in which the final concentration of DMSO is 2.5%. Medium control was prepared using sterile LB. Bacteria grew in LB medium at 37 °C and growth was detected as turbidity (560 nm) using a microtitre plate reader (Power Wave XS2, Biotek, VT, USA). The maximum level of DMSO to which the cells were exposed was 2.5%.

Biofilm formation assay

For biofilm quantification, a micro method based on a protocol previously reported was employed (O'Toole and Kolter 1998). Biofilms formed after 24 h incubation of bacterial cultures prepared as described in the previous paragraph, were stained with 20 μl of an aqueous solution of crystal violet (0.1%, w/v) for 20 min. After washing with water, the liquid was discarded from the wells and the material that remained fixed to the polystyrene (containing biofilm) was washed with PBS (thrice). Crystal violet bound to biofilm was removed from each well employing 200 μl absolute ethanol during 30 min at 37 °C with shaking. Absorbance (560 nm) of ethanol solutions of crystal violet was determined using a microtitre plate reader (Power Wave XS2, Biotek, VT, USA).

Ciprofloxacin, a known biofilm inhibitor, was incorporated in the bioassay at 5 μg/ml as a positive control in the same experimental conditions employed to evaluate the compounds. At this concentration, ciprofloxacin inhibit the biofilm formation but did not modify significantly the bacterial growth (Sandasi et al. 2011).

Quorum sensing inhibition assay

P. aeruginosa qsc 119 (reporter strain) is a mutant donated by P. Greenberg (Whiteley et al. 1999) that cannot produce its own AHLs (QS signal molecules). The reporter strain responds, by producing β-galactosidase, to exogenous active signal molecules generated by wild types *P. aeruginosa* strains. *P. aeruginosa* qsc 119 was constructed using a chromosomal promoter under the control of AHLs linked to lacZ. In consequence, β-galactosidase activity is under QS-control and in direct relationship with the autoinducers (AHLs) activity (Whiteley et al. 1999).

An overnight culture of the reporter strain grown at 37 °C in LB was diluted ten times in the same medium, reaching values of absorbance of 0.26 at $OD_{560\,nm}$. A 100 μl portion of this suspension was mixed, in each microplate well, with 100 μl cell-free culture supernatant obtained from *P. aeruginosa* ATCC 27853 cultured in LB media containing 200, 100, 50, 5 and 0.5 μg/ml of compounds **1**–**6**, during 24 h. Azithromycin, known to interfere with the QS process, was used at 5 μg/ml, concentration unable to affect the bacterial growth, as QS positive control under the same conditions as compounds **1**–**6** (Tateda et al. 2001). Control wells (8 replicates) contained cell-free culture supernatant (100 μl) obtained from *P. aeruginosa* ATCC 27853 cultured in LB media (190 μl) plus 10 μl of DMSO-water (1:1). β-Galactosidase activity was measured spectrophotometrically by Miller test (Miller 1972).

Elastase B activity

Elastolytic activity was determined using a modification of the method described by Caballero et al. (2001). Elastin Congo red (100 μl) (Sigma) dissolved in Tris–HCl (pH 8.0) at a concentration

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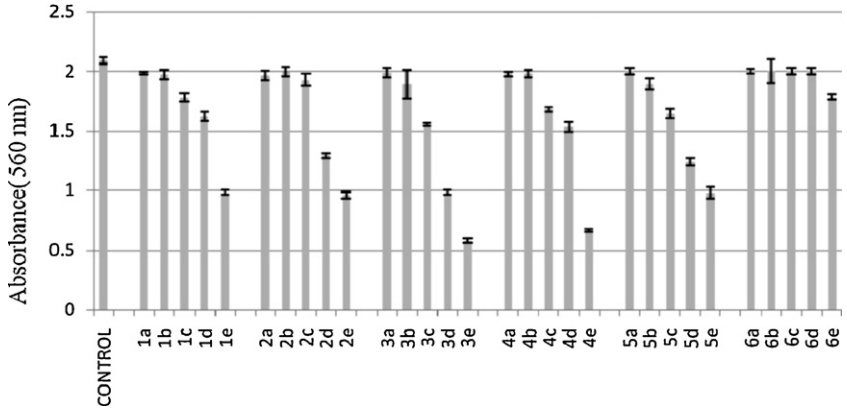

Fig. 2. Effect of sesquiterpene lactones on *P. aeruginosa* ATCC 27853 growth. Bacteria were grown in LB media with and without SLs isolated from *C. punctatum* at concentrations of 200 μg/ml (a), 100 μg/ml (b), 50 μg/ml (c), 5 μg/ml (d) and 0.5 μg/ml (e). Bacterial growth was assessed by reading the absorbance at 560 nm. Vertical bars represent the standard deviation ($n = 8$).

of 5 mg/ml was mixed with 100 μl cell-free culture supernatant from *P. aeruginosa* ATCC 27853 grown, during 24 h, in LB media containing 200, 100, 50, 5, and 0.5 μg/ml of SLs. The reaction mixture (200 μl) was incubated at 37 °C for 24 h and centrifuged at 13,000 rpm for 10 min. The absorbance (495 nm) of the supernatant is a measure of the enzyme activity.

Data analysis

Data are presented as mean ± SD. The statistical significance of differences between mean values was evaluated by *Student's test*. A value of $p \leq 0.05$ was considered significant.

Results

Bacterial growth

The effects of SLs on the bacterial growth (*P. aeruginosa* ATCC 27853) are shown in Fig. 2 in comparison with the control experiment. All the compounds assayed inhibit the bacterial growth at the highest concentration assayed; however, neither of them inhibits the bacterial growth at concentration of 5 μg/ml or lower. At 200 μg/ml the inhibitory effect of the SLs **1**, **2**, **3**, **4**, **5**, and **6** was 51, 53, 71, 67, 51, and 11% respectively. At 100 μg/ml the inhibitory effect was 26, 33, 52, 23, 28, and 0 respectively. Only compounds **1**, **3**, **4**, and **5** at 50 μg/ml inhibit bacterial growth 11, 23, 16, and 18% respectively.

Biofilm formation

The absorbance of biofilm (Fig. 3), formed after 24 h incubation in the control media, stained with crystal violet was 2.75. At 200 μg/ml SLs **1**–**6** inhibit 20, 30, 71, 31, 55 and 16% the biofilm production. At 100 μg/ml SLs **1**–**6** inhibit 13, 21, 60, 21, 40 and 13% the biofilm formation, respectively. However, at 50 μg/ml only compounds **2**, **3** and **5** inhibit significantly, 17, 50, and 31% the biofilm formation, respectively. Compounds **2**, **3** and **5** inhibit 11, 42, and 18% *P. aeruginosa* biofilm formation, respectively, even at the lower concentration (0.5 μg/ml). The IC_{50} for biofilm formation was 13 and 43 μmol/l for the compounds **3** and **5**, respectively.

Effects of SLs on QS

As shown in Fig. 4 at 200 μg/ml SLs **1**–**6** inhibit AHLs production 23, 50, 66, 55, 47, and 50%, respectively. At 100 μg/ml SLs **1**–**6** inhibit AHLs production 12, 39, 44, 42, 32, and 35% respectively. The IC_{50} for autoinducer production was 55, 37 and 45 μmol/l, for compounds **2**, **3** and **4**, respectively.

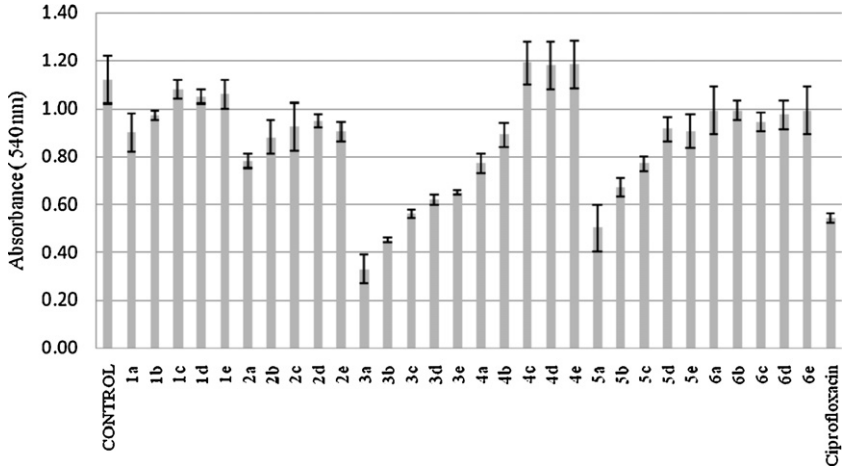

Fig. 3. Biofilm production of *P. aeruginosa* in the presence of SLs. Inhibition percentage of biofilm formation of *P. aeruginosa* cultures grown in LB broth untreated and treated with 200 μg/ml (a), 100 μg/ml (b), 50 μg/ml (c), 5 μg/ml (d) and 0.5 μg/ml (e) of SLs (**1**, **2**, **3**, **4**, **5**, and **6**) dissolved in DMSO/distilled water (50/50). Vertical bars represent the standard deviation ($n = 8$).

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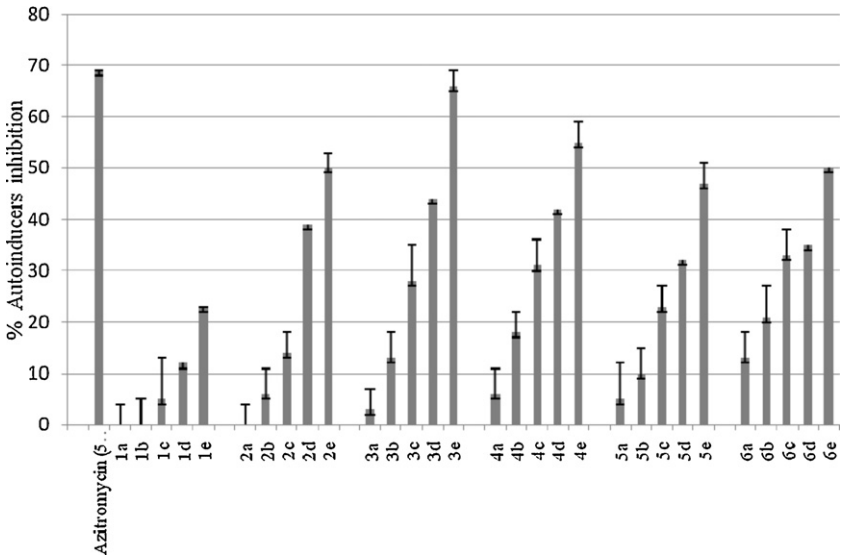

Fig. 4. Inhibition percentage of *P. aeruginosa* AHL production by SLs from *C. punctatum*. The inhibition percentage of calculated AHL, taking as reference the AHL production of *P aeruginosa* ATCC 27853 strain in LB broth (with 2.5% DMSO) without the addition of SLs. The results are the mean ($n = 4$). SL concentration used: 200 μg/ml (a), 100 μg/ml (b), 50 μg/ml (c), 5 μg/ml (d) and 0.5 μg/ml (e).

Elastase activity in culture supernatants

The elastase activity observed in culture supernatants incubated in the presence of all the SLs tested was inhibit even at 0.5 μg/ml (Fig. 5). Compounds **1**, **2**, **3**, **4**, **5**, and **6** produced 2, 22, 34, 51, 21 and 28% inhibition of elastase activity, respectively at 0.5 μg/ml. The IC_{50} for elastase activity was 6.3, 2.4, 0.6, 3.0 and 2.1 μmol/l for the compounds **2**, **3**, **4**, **5**, and **6**, respectively.

Discussion

Bacterial growth

The antibacterial activity of SLs was poor. However, the SLs goyazensolide-type (**2**, **3**, and **5**) showed higher bacterial growth inhibition than the SLs isogoyazensolide-type (**1**, **4**, and **6**). Nevertheless, in a previous paper using hole-plate diffusion in agar, compound **4** inhibited *Lactobacillus paracasei* ssp. *paracasei* CE 75, *L. plantarum* CE 105, and *L. plantarum* CE 358, *Staphylococcus aureus* F7; *S. aureus* ATCC 6538 P, and *Enterococcus faecalis* ATCC 39212 (Cartagena et al. 2008). In addition some SLs such as acanthospermal B, chlorojanerin and centaurepensin have bacteriostatic effect against *S. aureus* with a MIC of 50, 16 and 64 μg/ml, respectively (Arena et al. 2011; Özçelik et al. 2007). Moreover, thirty SLs exhibited antibacterial activity against Gram positive bacteria but no against Gram negative strains (Cartagena et al. 2008), as was previously pointed out for medicinal plant extracts, as well as some natural products (Cowan 1999; Smith et al. 2007).

Effects of SLs on biofilm

The percentage of biofilm inhibition by SLs was higher than the percentage of growth inhibition by the same compounds. The relation between biofilm production (measured at $DO_{540\,nm}$)/bacterial growth (measured at $DO_{560\,nm}$) was defined as specific biofilm produced, *i.e.*, biofilm each bacterium forms. The specific biofilm

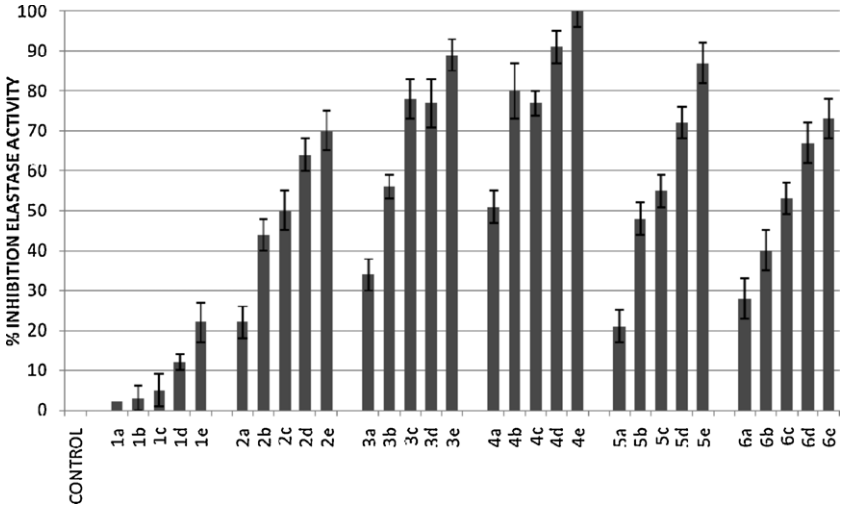

Fig. 5. Elastase activity percentage of supernatant of *P. aeruginosa* incubated 24 h in the presence of SLs isolated from *C. punctatum*. The results are the mean ($n = 4$) (standard deviation). SL concentration used: 200 μg/ml (a), 100 μg/ml (b), 50 μg/ml (c), 5 μg/ml (d) and 0.5 μg/ml (e).

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production for the control media was 0.53.When it is lower than control, it shows that specific production is lower than that of control. Under the conditions studied compounds **2**, **3**, and **5** tend to reduce biofilm specific production. The specific biofilm production was 0.46, 0.33, 0.45 for the compounds **2**, **3** and **5**, respectively at 0.5 μg/ml.

Increasing evidence is showing that the ability to form biofilms in many organisms involves QS regulation. Compounds extracted from plants or their essential oils could inhibit bacterial growth or affect biofilm formation and structure (Fonseca and Sousa 2007; Costerton et al. 1999). In fact, the biofilm inhibition by SLs **2**, **3** and **5** is clearly related to the inhibition of AHLs production.

Effects of SLs on AHLs production

The reduction of the autoinducer (AHL) concentrations in bacterial cultures treated with SLs could be the result of inhibition of cell growth, or inhibition of AHLs production. With regard to the first hypothesis, it was observed that microbial growth in the strain cultures untreated and treated with SLs was similar. With respect to the second hypothesis, we demonstrated that SLs reduce AHLs production in *P. aeruginosa*.

Effects of SLs on elastase activity

It is well known that elastase activity is controlled by AHLs. We analyzed the elastase activity in relation to the concentration of autoinducers, affected by our SLs. All the remaining lactones affect elastase activity and AHLs formation as well, but not at the same rates, indicating that SLs **2**–**6** also act by other mechanisms to affect elastase activity.

The results presented here indicate that SLs interfere with QS, which has an essential role in biofilm maturation and in elastase activity. Given the large number of pathogenic bacteria that utilize these mechanisms of action (de Kievit and Iglewski 2000); natural, small molecular weight compounds may find applications in drug discovery. The biofilm inhibition displayed by compounds **2**, **3** and **5** is comparable with that reported for other sesquiterpene lactones from higher plants (Cartagena et al. 2007) while the effects of the same compounds on the autoinducers production are at the same level of the strongest QS inhibitors reported so far (Castang et al. 2004). In the current study, we have identified at least three natural products (**2**, **3**, and **5**) from *C. punctatum* that have biological activity against the pathogenic properties of *P. aeruginosa* attenuating biofilm formation as well as down-regulating elastase activity and QS (AHLs) autoinducer production.

In conclusion, we have identified at least three natural products (**2**, **3**, and **5**) from *C. punctatum* that have biological activity against the pathogenic properties of *P. aeruginosa*.

Acknowledgements

The authors thank CIUNT (26-D 410 and 407) and ANCyT (PICT 34918/05 and 816/06) for their grants.

References

Adonizio, A.L., Downum, K., Bennett, B.C., Mathee, K., 2006. Anti-quorum sensing activity of medicinal plants in southern Florida. Journal of Ethnopharmacology 105, 427–435.

Álvarez Valdés, D., Bardón, A., Catalán, C.A.N., Gedris, T.E., Herz, W., 1998. Goyazensolides and isogoyazensolides from Argentine *Centratherum punctatum* ssp. *punctatum*. Biochemical Systematics and Ecology 26, 805–808.

Arena, M.E., Cartagena, E., Gobbato, N., Baigori, M., Valdez, J.C., Bardon, A., 2011. *In vivo* and *in vitro* antibacterial activity of acanthospermal B, a sesquiterpene lactone isolated from *Acanthospermum hispidum*. Phytotherapy Research 25, 597–602.

Caballero, A.R., Moreau, J.M., Engel, L.S., Marquart, M.E., Hill, J.M., O'Callaghan, R.J., 2001. *Pseudomonas aeruginosa* protease IV enzyme assays and comparison to other *Pseudomonas* proteases. Analytical Biochemistry 290, 330–337.

Cartagena, E., Colom, O.Á., Neske, A., Valdez, J.C., Bardón, A., 2007. Effects of plant lactones on the production of biofilm of *Pseudomonas aeruginosa*. Chemical and Pharmaceutical Bulletin 55, 22–25.

Cartagena, E., Montanaro, S., Bardón, A., 2008. Improvement of the antibacterial activity of sesquiterpene lactones. Revista Latinoamericana de Química 36, 43–51.

Castang, S., Chantegrel, B., Deshayes, C., Dolmazon, R., Gouet, P., Haser, R., Reverchon, S., Nasser, W., Hugouvieux-Cotte-Pattat, N., Doutheau, A., 2004. N-sulfonyl homoserine lactones as antagonists of bacterial quorum sensing. Bioorganic and Medicinal Chemistry Letters 14, 5145–5149.

Cathcart, G.R., Gilmore, B.F., Greer, B., Harriott, P., Walker, B., 2009. Inhibitor profiling of the *Pseudomonas aeruginosa* virulence factor LasB using N-alpha mercaptoamide template-based inhibitors. Bioorganic and Medicinal Chemistry Letters 19, 6230–6232.

Costerton, J.W., Stewart, P.S., Greenberg, E.P., 1999. Bacterial biofilms: a common cause of persistent infections. Science 284, 1318–1322.

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

Cumberbatch, A., 2002. Characterization of the anti-quorum sensing activity exhibited by marine macroalgae of South Florida. In: Mathee, K. (Mentor), Undergraduate Honors Thesis. Florida International University.

de Kievit, T.R., Iglewski, B.H., 2000. Bacterial quorum sensing in pathogenic relationships. Infection and Immunity 68, 4839–4849.

Fonseca, A.P., Sousa, J.C., 2007. Effect of shear stress on growth, adhesion and biofilm formation of *Pseudomonas aeruginosa* with antibiotic-induced morphological changes. International Journal of Antimicrobial Agents 30, 236–241.

Fuqua, W.C., Winans, S.C., Greenberg, E.P., 1994. Quorum sensing in bacteria: the LuxR–LuxI family of cell density-responsive transcriptional regulators. Journal of Bacteriology 176, 269–275.

Gao, M., Teplitski, M., Robinson, J.B., Bauer, W.D., 2003. Production of substances by *Medicago truncatula* that affect bacterial quorum sensing. Molecular Plant–Microbe Interactions 16, 827–834.

Gilabert, M., Ramos, A.N., Schiavone, M.M., Arena, M.E., Bardón, A., 2011. Bioactive sesqui- and diterpenoids from the Argentine liverwort *Porella chilensis*. Journal of Natural Products 74, 574–579.

Liu, P.V., 1974. Extracellular toxins of *Pseudomonas aeruginosa*. Journal of Infectious Diseases 130, S94–S99.

Miller, J.H., 1972. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Özçelik, B., Gürbüz, I., Karaoglu, T., Yeşilada, E., 2007. Antiviral and antimicrobial activities of three sesquiterpene lactones from *Centaurea solstitialis* L. ssp. *solstitialis*. Microbiological Research 164, 545–552.

O'Toole, G.A., Kolter, R., 1998. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. Molecular Microbiology 28, 449–461.

Otto, M., 2004. Quorum-sensing control in *Staphylococci* – a target for antimicrobial drug therapy? FEMS Microbiology Letters 241, 135–141.

Rice, S.A., Mc Dougald, D., Kumar, N., Kjelleberg, S., 2005. The use of quorum-sensing blockers as therapeutic agents for the control of biofilm-associated infections. Current Opinion in Investigational Drugs 6, 178–184.

Sandasi, M., Leonard, C.M., Van Vuuren, S.F., Viljoen, A.M.S., 2011. The in vitro antibiofilm activity of selected culinary herbs and medicinal plants against *Listeria monocytogenes*. African Journal of Botany 77, 80–85.

Smith, J.E., Tucker, D., Watson, K., Jones, G.L., 2007. Identification of antibacterial constituents from the indigenous Australian medicinal plant *Eremophila duttonii* F. Muell. (Myoporaceae). Journal of Ethnopharmacology 112, 386–393.

Socolsky, C., Arena, M.E., Asakawa, Y., Bardón, A., 2010. Antibacterial prenylated acylphloroglucinols from the fern *Elaphoglossum yungense*. Journal of Natural Products 73, 1751–1755.

Sokol, P.A., Kooi, C., Hodges, R.S., Cachia, P., Woods, D.E.J., 2000. Inhibitors of this virulence factor have previously been demonstrated to protect host tissue and reduce the virulence of infections. Infectious Diseases 181, 1682–1686.

Stewart, P., Costerton, J., 2001. Antibiotic resistance of bacteria in biofilms. Lancet 358, 135–138.

Tateda, K., Comte, R., Pechere, J.C., Kohler, T., Yamaguchi, K., Van Delden, C., 2001. Azithromycin inhibits quorum sensing in *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy 45, 1930–1933.

Teplitski, M., Robinson, J.B., Bauer, W.D., 2000. Plants secrete substances that mimic bacterial *N*-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. Molecular Plant–Microbe Interactions 13, 637–648.

Whiteley, M., Lee, K.M., Greenberg, E.P., 1999. Identification of genes controlled by quorum sensing in *Pseudomonas aeruginosa*. Proceedings of the National Academy of Sciences of the United States of America 96, 13904–13909.

Zhang, L.H., Dong, Y.H., 2004. Quorum sensing and signal interference: diverse implications. Molecular Microbiology 53, 1563–1571.

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