Volatile Terpenoids from Water Pepper (*Polygonum punctatum*) Against *Pseudomonas aeruginosa* and *Staphylococcus aureus* Virulence Strategies

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Abstract: *Polygonum punctatum* Elliot (water pepper) is a pungent herb ancestrally employed as a disinfectant in traditional medicine by Toba Indians of the north-eastern region of Argentina and also commonly used as spice in Japanese cuisine. GC-MS of whole diethyl ether extract (EE) from aerial parts allowed to identify 14 volatile terpenoids such as sesquiterpenes: *α*-bisabolol (3.4 %), polygodial and isotadeonal (34.0%); various methylated phenol like *α*-tocopherol or vitamin E (3.6 %), and phytosterols: stigmasterol (2.1%) and *β*-sitosterol (29.9 %). Thus, water pepper is a promising source of drimane sesquiterpenes and phytoestrogens with important bioactivities.

Following a taste- guided fractionation by CC and HPLC, drimane-type sesquiterpenes, polygodial (**1**) and its stereoisomer isotadeonal (**2**) were isolated as main compounds from the EE. The antipathogenic effects on the bacterial growth, biofilm formation, and elastase activity of both pure compounds and EE were evaluated against two *Staphylococcus aureus* and two *Pseudomonas aeruginosa* strains at 10 and 100 µg/mL.

The highest effects were observed for the non pungent drimane isotadeonal (**2**) which was able to reduce about 75 % the bacterial growth of all tested microorganisms and to inhibit Gram-positive biofilm formation (85 % mean) at 100 µg/mL. In addition, elastase activity of *P. aeruginosa*, another virulence strategy, was attenuated more than 50 % at 100 µg/mL by **2**.

These results provide evidence that support the antimicrobial use of *P. punctatum* against *P. aeruginosa* and *S. aureus*, as well as, demonstrating that isotadeonal (**2**), despite it has been suggested to lack biological properties, is a bioactive compound able to control biofilm formation and bacterial growth of both human pathogens.

Keywords: Isotadeonal, biofilm, *Polygonum punctatum*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

1. INTRODUCTION

The genus *Polygonum* (Polygonaceae), which comprises about 300 species [1], is worldwide distributed in temperate climates and it is represented in Argentina by 21 species [2]. It is a cosmopolitan plant that grows in damp places and shallow water of America, Australia, New Zealand, temperate Asia and Europe. The *Polygonum* genus is well known for producing a variety of secondary metabolites including flavonoids, triterpenoids, anthraquinones, coumarins, phenylpropanoids, tannins, and drimanes [1,3].

The unsaturated dialdehyde (-)-polygodial, which posses a drimane backbone, has been found to be responsible for the pungent taste of some species of the genus, and hence for its uses as a spice. Polygodial is also a component of the “hot” taste in peppery spices common in traditional Japanese cuisine. In addition, polygodial is one of the main bioactive compounds in many of the plants used in traditional medicine in Japan, China, Africa, and Brazil [4]. Furthermore, it has proven that is capable of acting against a notable variety of bacteria and fungi such as S*accharomyces cerevisiae, Candida albicans,* and *Salmonella* [5,6]. This drimane compound has been reported for five species of the *Polygonum* genus: *P. punctatum, P. hydropiper*, *P. acuminatum,* and *P. persicaria,* and it was recognized as a key molecule that plays an important role in plants defense against predators, being fungitoxic, antifeedant against insects, as well as inhibiting the transmission of some viruses [2,7].

Particularly, *P. punctatum* (water pepper, ajicillo, erva do bicho, caa-tai) which is used as a disinfectant in traditional medicine by Toba Indians of the north-eastern region of Argentina [8], demonstrated to be effective against a broad spectrum of microorganisms that includes *Staphylococcus aureus, Bacillus subtilis*, *Micrococcus luteus*, *Aspergillus niger* and *Mucor sp*. [9].

Bacterial biofilms are complex communities of bacteria embedded in a self-produced matrix and

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attached to inert or living surfaces [10]. These microorganism communities are more resistant to the immune system and to antibiotics than planktonic or free-floating bacteria. There are a few studies in which chemical compounds with a strong biofilm-specific inhibition were identified [11]. A previous report indicates that drimane-type sesquiterpenes can be promising substances to biofilm formation control [12].

Pseudomonas elastase is a metalloprotease also known as pseudolysin or LasB which has long been recognized as a key virulence factor produced by *P. aeruginosa* [13]. LasB acts within the bacterial cell as a regulator in the generation of the secreted polysaccharides that constitute the bacterial biofilm. In addition, this enzyme degrades a broad range of host tissue proteins and biomolecules involved in innate immunity such as immunoglobulins, complement factors and cytokines. Thus LasB inhibition could be important for attenuation of *Pseudomonas* virulence [14].

Herein, volatile compounds from an Argentinean collection of *Polygonum punctatum* were investigated. Following a taste- guided fractionation, drimane-type sesquiterpenes were isolated as main compounds from a diethyl ether extract (EE) of this spicy herb. Antipathogenic properties of EE and its drimane-type sesquiterpene dialdehydes, polygodial (**1**) and its non-pungent stereoisomer isotadeonal (known as isopolygodial, **2**), were determined against four pathogenic bacteria. EE and **1** and **2** were screened in their ability to control bacterial growth, biofilm formation, and elastase activity of two strains of *P. aeruginosa*, one of them from a clinically isolated. Furthermore, antibacterial and antibiofilm activities were measured on two strains of *S. aur*eus (one of them methicillin-resistant).

2. MATERIALS AND METHODS

2.1. General

GC analysis was carried out using a TRACE GC chromatograph (Thermo Scientific, TX, USA). Low-resolution MS spectra were recorded using a POLARIS Q instrument (Thermo Scientific, TX, USA). NMR spectra were measured in $CDCl_3$ at 300 MHz on a Bruker 300 spectrometer. The HPLC separations were performed on a Gilson apparatus, using a silica gel column Chemo Pack Develosil 60 (5 µm, 10 mm i.d. x 250 mm) and a refractive index detector.

2.2. Plant Material

P. punctatum Elliot was collected in the Yungas region of Tucumán, Argentina. The air-dried aerial parts (119.0 g) were extracted at room temperature with agitation for 14 days with diethyl ether to give 6.0 g of residue after the solvent removal in a rotary evaporator (yield of 5 %). The EE was subjected to Sephadex LH20 CC (MeOH-CH_2Cl_2, 1:1), silica gel CC (70-230 mesh) with *n*-hexane and increasing amounts of EtOAc (0-100%), and finally MeOH as eluents. The elution was monitored using TLC on aluminium-precoated plates with F254 indicator. The spots on the plates were visualised under UV light, and the plates were then sprayed with Godin reagent. It is known that polygodial has a similar retention factor (RF) in hexane-EtOAc 85:15 as cholesterol, and for this reason, cholesterol was used as a polarity reference. The eluates were tasted for their pungency. Fractions that eluted with a RF similar to cholesterol and with a pungent taste were combined, and this mixture was further processed using normal-phase HPLC (hexane-EtOAc17:3 at 1.5 mL/min).

2.3. Microorganisms

Four pathogenic bacteria were studied. Two of them were *Staphylococcus aureus* strains, and the other two *Pseudomonas aeruginosa*. *S. aureus* strains were ATCC 6538 P and *S. aureus* methicillin-resistant F7; while *P. aeruginosa strains* were *P. aeruginosa* RPA100, isolated from an active infectious process (Collection of Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Argentina), and *P. aeruginosa* ATCC 27853.

2.4. Bacterial Growth

Overnight cultures of *P. aeruginosa* and *S. aureus* were diluted to reach an OD of 0.125 ± 0.01 at 600 nm in Luria–Bertani (LB) and Mueller-Hinton (MH) media, respectively. A 180 µL aliquot of the diluted culture was placed in wells of a plastic microtitre plate. Solutions containing 0.1 and 0.01 mg/mL of EE, **1**, and **2** in a DMSO: distilled water mixture (1:1) were prepared separately, and 20 µL of each solution was pipetted to the polystyrene microtitre plate wells individually (8 replicates). Negative control wells (8 replicates) contained 180 µL of the diluted culture with 20 µL of DMSO: water (1:1). The bacterial growth was detected as turbidity at 600 nm using a microtitre plate reader (Power Wave XS2; BioTek, Vermont, USA).

2.5. Biofilm Formation Assay

For biofilm quantification, a micro-method based on a previously reported protocol was employed [15]. 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. Then, the liquid was discarded from wells and the material that remained fixed to the plastic-polystyrene (containing biofilm) was washed with PBS (thrice). Crystal violet bound to biofilm was removed from each well employing 200 µL of absolute ethanol during 30 min at 37°C with shaking. Absorbance (540 nm) of ethanol solutions of crystal violet was determined using a microtitre plate reader (Power Wave XS2. Biotek, Vermont, USA).

2.6. Elastase B Activity

Elastolytic activity was determined using a modification of a previously reported method [16]. Elastin Congo red (100 µL) (Sigma) dissolved in Tris–HCl (pH 8.0) at a concentration of 5 mg/mL was mixed with 100 µL cell-free culture supernatant from *P. aeruginosa* strains grown, during 24 h, in LB media containing 100 µg/mL of EE, polygodial, and isotadeonal. 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.

2.7. Statistical Analysis

The experimental data were subjected to the analysis of variance (ANOVA) and the separation of means with least significance difference (LSD), using the One Way ANOVA and the parametric T test of Student-Newman-Keuls at 95% level of confidence.

3. RESULTS AND DISCUSSION

3.1. Chemical Analysis

GC-MS analysis of the EE from aerial parts of *P. punctatum* has led to the identification of 14 plant volatile terpenoids (Table **1**) such as sesquiterpenes: *α*-bisabolol (3.4 %), polygodial and isotadeonal (34.0%); various methylated phenol as *α*-tocopherol or vitamin E (3.6 %), and phytosterols: stigmasterol (2.1%) and *β*-sitosterol (29.9 %). Thus water pepper is a promising source of drimane sesquiterpenes and phytoestrogens that containing important bioactivities.

The EE was further subjected to CC and HPLC combined methodologies to afford the drimane-type sesquiterpenes polygodial (**1**) and isotadeonal (**2**). Compounds were identified by their spectroscopic features after comparison with the available data in literature [2,7,17].

When purified polygodial was analyzed by GC-MS two peaks were detected, one of the detected picks was identical to isotadeonal and the other one was

Table 1: GC-MS of *Polygonum punctatum* Constituents

Compounds	Formula	RT (min)	Area (%)
β-Selinene	$C_{15}H_{24}$	22.9	1.5
β-Caryophillene	$C_{15}H_{24}$	24.2	1.7
Cis-α-bisabolene	$C_{15}H_{24}$	25.7	0.1
Germacrene D	$C_{15}H_{24}$	26.8	0.2
γ-maaliene	$C_{15}H_{24}$	27.4	0.5
Cedrene	$C_{15}H_{24}$	28.5	2.4
Caryophillene oxide	$C_{15}H_{24}O$	31.0	0.3
Spathulenol	$C_{15}H_{24}O$	33.1	0.3
Agarospirol	$C_{15}H_{26}O$	34.0	0.2
α-Bisabolol	$C_{15}H_{26}O$	34.9	3.4
Polygodial and isotadeonal	$C_{15}H_{22}O_2$	42.7- 46.4	34.0
α-Tocopherol	$C_{29}H_{50}O_2$	60.6	3.6
Stigmasterol	$C_{29}H_{48}O$	68.4	2.1
β-Sitosterol	$C_{29}H_{50}O$	71.6	29.9

identical to the original polygodial, thus the appearance of isotadeonal into the GC analysis of polygodial is probably an artifact. For this reason, in the total ion current chromatogram (TIC) of whole EE was impossible to determine the area relationships between both isomers [18].

Previous reports have suggested that isotadeonal (**2**) could be an artifact produced during the isolation process, and therefore it shouldn't be considered as a natural product [19]. However, in the present research was possible to detect the presence of both isomers into the EE by NMR before the application of any chromatographic process. This result is in agreement with previous studies that clearly indicate that **2** is a natural product. Indeed, isotadeonal was isolated as natural product from *Polygonum hydropiper* L. [20], *Warburgia salutaris* [17], and *Capsicodendron dinisii* [21]. Concentration ratios of isotadeonal/polygodial appear to be influenced by the vegetal species and collection time [22].

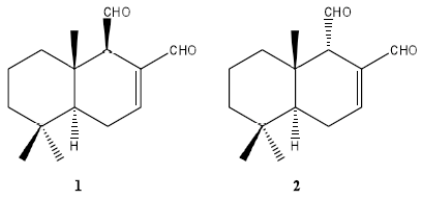

Figure 1: Chemical structures of drimanes from *Polygonum punctatum.* **1** – Polygodial, **2** – Isotadeonal (Isopolygodial).

3.2. Antibacterial Activity

P. aeruginosa ATCC 27853 bacterial growth was inhibited by **1** (polygodial), **2** (isotadeonal), and EE at 100 µg/mL (22, 68, and 66 %, respectively), as shown in Figure **2**. While, **2** and **1** at 10 µg/mL gave a bacterial growth inhibition of 21 and 7 %, respectively (data not shown). The *P. aeruginosa* multi-resistant strain growth was also inhibited by **2** and EE (86 and 35 %, respectively) at 100 µg/mL. Neither performed tests showed bacterial growth inhibition at 10 µg/mL (data not shown).

Compound **2** (100 µg/mL) was also the most active drimane against *S. aureus*, which inhibited by 82% of *S. aureus* ATCC 6538 P growth. While, EE inhibited by 67 %. Compound **1**, also reduced the cell growth but only a 18% after 24 h incubation with respect to control, at the same concentration (Figure **2**). Whereas, *S. aureus* methicillin-resistant F7 growth was inhibited 80% and 62 % by **2** and EE, respectively. None of the treatments was able to inhibit *S. aureus* growth at 10 µg/mL (data not shown).

It is important to note that **2** was able to produce a strong growth inhibition against all bacterial strains of this bioassay, while compound **1** only faintly reduced bacterial growth of ATCC strains, and did not significantly modify the bacterial growth of the resistant strains.

3.3. Biofilm Inhibition

Absorbance measurements of biofilm formed after 24 h incubation are shown in Figure **3**. *P. aeruginosa* ATCC 27853 biofilm was inhibited by 26, 59, and 24 % with 100 µg/mL of **1**, **2**, and EE, respectively. While, *P. aeruginosa* multi-resistant strain's biofilm was inhibited by 19, 63, and 31 % in presence of **1**, **2**, and EE,

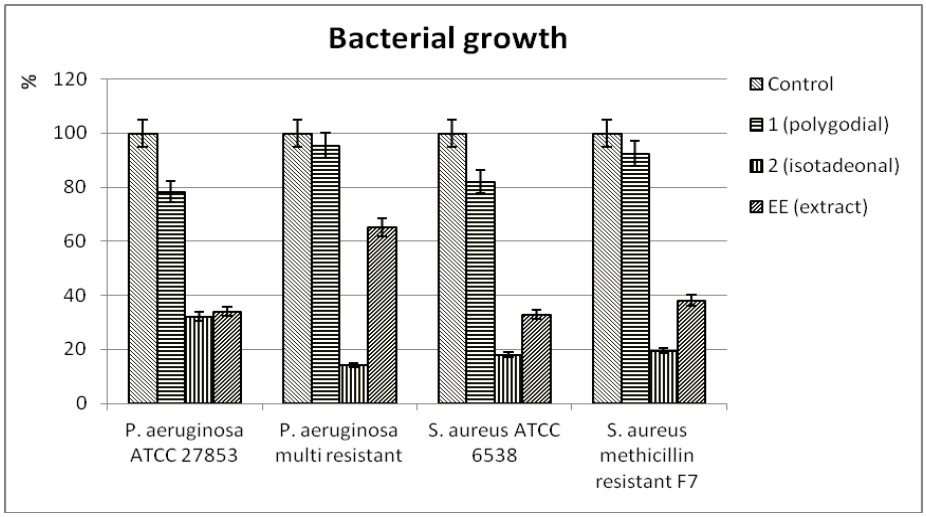

Figure 2: Effects of compounds **1** (polygodial), **2** (isotadeonal), EE (ethyl ether extract of *Polygonum punctatum*) at 100 µg/mL, and negative control on *Pseudomonas aeruginosa* ATCC 27853, *P. aeruginosa* muti-resistant, *Staphylococcus aureus* ATCC 6538 P, and *S. aureus* F7 growth in Moeller Hinton medium.

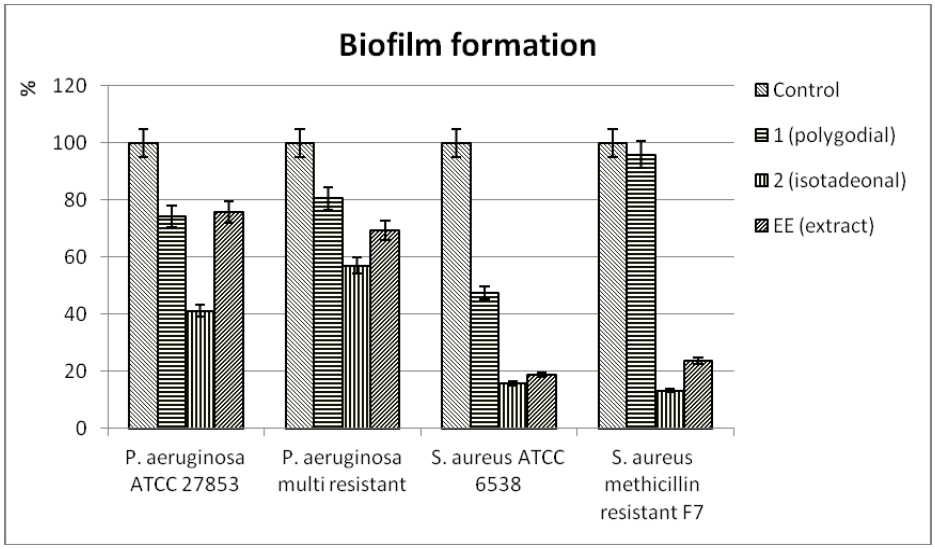

Figure 3: Effects of compounds **1** (polygodial), **2** (isotadeonal), EE (ethyl ether extract of *Polygonum punctatum*) at 100 µg/mL, and negative control on *Pseudomonas aeruginosa* ATCC 27853, *P. aeruginosa* muti-resistant, *Staphylococcus aureus* ATCC 6538 P, and *S. aureus* F7 biofilm formation measured as optical density at 540 nm after crystal violet staining in a microtitre plate test.

respectively (at the same concentration). Lower effects at 10 µg/mL of compounds or EE against *P. aeruginosa* ATCC 27853 were observed (about 30 %). None of the tested compounds or EE was able to inhibit significantly the *P. aeruginosa* multi-resistant biofilm at 10 µg/mL (data not shown).

S. aureus ATCC 6538 P biofilm formation decreased by 53, 84, and 81% in presence of 100 µg/mL of **1**, **2**, and EE, respectively (Figure **3**). As well as, the *S. aureus* F7 biofilm formation was reduced by 87 and 76 %, in presence of 100 µg/mL of **2** and EE, while polygodial (**1**) did not produce significant biofilm inhibition. Only EE at 10 µg/mL was able to produce slight biofilm formation inhibition (about 30 %) for both *S. aureus* strains (data not shown).

3.4. Elastase B Inhibition

Compounds **1**, **2**, and EE also inhibited the elastase B activity of *P. aeruginosa* ATCC 2785 by 48, 76 and 52 % at 100 µg/mL, and 18, 33, and 19 % at 10 µg/mL, respectively. As well as, *P. aeruginosa* multi-resistant elastase activity decreased 24, 56, and 37 % in presence of 100 µg/mL of **1**, **2**, and EE, respectively. At

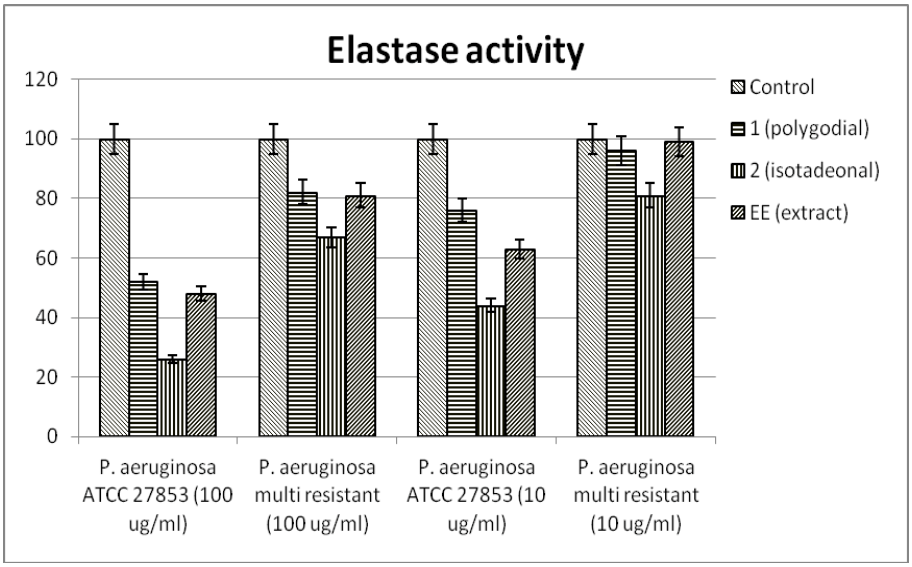

Figure 4: Effects of compounds **1** (polygodial), **2** (isotadeonal), EE (ethyl ether extract of *Polygonum punctatum*) at 100 µg/mL and 10 µg/mL, and negative control on *Pseudomonas aeruginosa* ATCC 27853 and *P. aeruginosa* muti-resistant elastase activity measured as optical density at 495 nm.

10 µg/mL, only isotadeonal (**2**) was able to inhibit elastase activity (19 %), as shown in Figure **4**.

Previous reports on the effects of polygodial against fungi established that its mechanism of action involve membrane disruption [23] and mitochondrial ATP synthase enzyme inhibition [24]. However, only a few studies about *Polygonum* compounds against pathogenic bacteria were carried out. The activity showed by the related specie *P. cuspidatum*, traditionally used in folk medicine to control oral diseases, against bacterial biofilm does not involve drimane compounds. A *P. cuspidatum* column fraction containing anthraquinones, cardiac glycosides, and phenolics compounds inhibits the biofilm of dental bacteria *Streptococcus mutans* and *Streptococcus sobrinus* [25].

It was previously reported that medicinal plant extracts, as well as some natural products, that displayed antibiotic activity against Gram-positive bacteria, lacked activity against Gram (-) bacteria [26,27]. It is worthy to point out that polygodial (**1**) and isotadeonal (**2**) were active against both, Gram-positive and negative bacteria, although they were more effective against Gram-positive strains.

As stated in previous reports [28], for both *S. aureus* and *P. aeruginosa*, ATCC isolates were more susceptible to the tested natural products than the clinical isolates. In these, the biofilm formation appears to provide a higher protection; e.g., polygodial inhibited the *S. aureus* ATCC 6538 P biofilm by 53 % at 100 µg/mL, while for the methicillin-resistant strain F7, polygodial did no significantly modify the biofilm formation at the same concentration. Similar effects were observed for *P. aeruginosa.*

Among the tested compounds, isotadeonal (**2**) was the most active inhibitor, by reducing about 75 % bacterial growth of all tested microorganisms. This may be indicative of a broad-spectrum antibiotic activity. In addition, **2** inhibited 85 % (mean) Gram-positive strains' biofilms, and decreased the Gram-negative strains' elastase activity more than 50 %. The EE also showed an interesting activity, which can be explained not only by the important amounts of the active drimanes isotadeonal and polygodial in the EE, but also by the occurrence of spathulenol (see Table **1**), a sesquiterpene that has proven to have antibacterial and anti-quorum sensing activity [29].

The important activity showed by isotadeonal adds new evidence to the fact reported by Anke and Sterner [30] and Derita *et al.* [22], who determined that in contrast with previous suggestions [23] isotadeonal is clearly a bioactive compound, and more studies are needed to further characterize its full range of antibiotic and anti-pathogenic properties.

Given that drimanes from *P. punctatum* were effective to control bacterial growth of some human pathogenic bacteria strains, the results reported here can partially explain the popular use of *P. punctatum* as antiseptic by the Toba indigenous population. In addition, due to *S. aureus* is the main food spoilage, and the presence of *P. aeruginosa* in many kind of food is well reported [28,31], microbial biofilm inhibition is an important target in the field of food conservation. Then, besides of the potential uses of pure spicy compounds as antimicrobials in human infections, the not spicy drimanes act as the main active compounds suggesting that the uses of *P. punctatum* as spice can provide beneficial as a food conservative. We report here for the first time the antibiofilm and anti-elastolytic properties of *P. punctatum* against *S. aureus* and *P. aeruginosa*, and demonstrated that isotadeonal (**2**), which was generally considered biologically inactive, is a strong inhibitor able to control biofilm formation and bacterial growth of both tested kinds of bacteria. The results of this study reinforce the anciet use of the herb as antimicrobial in food

3.5. Polygodial (1)

^{1}H NMR (300 MHz, $CDCl_3$): 9.48 (1H, *d*, *J*= 4.2 Hz, H-11); 9.42 (1H, *s*, H-12); 7.11 (1H, *m*, H-7); 2.78 (1H, *dddd*, *J*= 6.2, 2.1, 2.1, 2.1 Hz, H-9); 2.55-2.40 (1H, *m*, H-6α); 2.35-2.20 (1H, *m*, H-6β); 1.82 (1H, *m*, H-1β); 1.54-1.43 (3H, *m*, H-2α, 2β, 3β); 1.34 (1H, *td*, *J*= 4.0 and 13.4 Hz, H-1α); 1.26-1.16 (2H, *m*, H-3α, H-5); 0.92; (3H, *s*, Me-15); 0.91 and 0.89 (6H, 2*s*, Me-14 and Me-15). ^{13}C NMR (75 MHz, $CDCl_3$): 201.9 (HC=O); 193.2 (HC=O); 154.4 (=CH); 138.1 (=C); 60.2 (CH); 48,8 (CH); 41.7 (CH_2); 39.5 (CH_2); 36.8 (C); 33.0 (C); 33.0 (CH_3); 25.1 (CH_2); 21.9 (CH_3); 17.9 (CH_2); 15.2 (CH_3). MS (EI, 70 eV): *m/z* (%) = 234 [M^+], 216 [M^+ - H_2O], 206 [M^+ - CO], 191 [206 - Me].

3.6. Isotadeonal (2)

^{1}H NMR (300 MHz, $CDCl_3$): 9.86 (1H, *d*, *J*= 2.7 Hz, H-11); 9.41 (1H, *s*, H-12); 7.11 (1H, *dd*, *J*= 2.7 and 4.9 Hz, H-7); 3.26 (1H, *bm*, H-9); 2.57 (1H, *dt*, *J*= 5.0, 5.0, 20.6 Hz, H-6α); 2.22 (1H, *dddd*, *J*= 1.8, 2.6, 11.6 and 20.6 Hz, H-6β); 1.80 (1H, *m*, H-1β); 1.62-1.52 (5H, *m*, H-1α, 2α, 2β, 3β, 5); 1.19 (1H, *ddd*, *J*= 0.62, 4.1 and

12.0 Hz, H-3α); 0.97 (3H, *s*, Me-15); 0.94 and 0.92 (6H, 2*s*, Me-13 and Me-14). ^{13}C NMR (75 MHz, $CDCl_3$): 202.2 (HC=O); 192.8 (HC=O); 153.5 (=CH); 137.3 (=C); 58.5 (CH); 44.2 (CH); 42.0 (CH_2); 37.6 (CH_2); 37.1 (C); 32.9 (C); 32.7 (CH_3); 25.5 (CH_2); 21.9 (CH_3); 21.5 (CH_2); 18.4 (CH_3). MS (EI, 70 eV): *m/z* (%) = 234 [M^+], 216 [M^+ - H_2O], 206 [M^+ - CO], 191 [206 - Me].

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REFERENCES

[1] Wang KJ, Zhang YJ, Yang CR. Antioxidant phenolic compounds from rhizomes of *Polygonum paleaceum*. J Ethnopharmacol 2005; 96: 483-7. http://dx.doi.org/10.1016/j.jep.2004.09.036

[2] Derita MG, Gattuso SJ, Zacchino SA. Occurrence of polygodial in species of *Polygonum* genus belonging to Persicaria section. Biochem Syst Ecol 2008; 36: 55-8. http://dx.doi.org/10.1016/j.bse.2007.05.011

[3] López SN, Sierra MG, Gattuso SJ, Furlán RL, Zacchino SA. An unusual homoisoflavanone and a structurally-related dihydrochalcone from *Polygonum ferrugineum* (Polygonaceae). Phytochemistry 2006; 67: 2152-8. http://dx.doi.org/10.1016/j.phytochem.2006.06.018

[4] Jonassohn M. Sesquiterpenoids unsaturaded dialdehydes. Ph D Thesis, Lunde University 1996.

[5] Kubo I, Fujita K, Lee SH. Antifungal mechanism of polygodial. J Agric Food Chem 2001; 49: 1607-11. http://dx.doi.org/10.1021/jf000136g

[6] Kubo I, Fujita KI, Lee SH, Ha TJ. Antibacterial activity of polygodial. Phytother Res 2005; 19: 1013-7. http://dx.doi.org/10.1002/ptr.1777

[7] Alves TMDA, Ribeiro FL, Kloos H, Zani CL. Polygodial, the Fungitoxic Component from the Brazilian Medicinal Plant *Polygonum punctatum*. Mem I Oswaldo Cruz 2001; 96: 831-3. http://dx.doi.org/10.1590/S0074-02762001000600016

[8] Martínez-Crovetto R, Las plantas utilizadas en medicina popular en el noroeste de Corrientes (República Argentina), Ministerio de Cultura y Educación, Tucumán, Argentina, 1981.

[9] Penna C, Marino S, Vivot E, Cruañes MC, De D, Muñoz J, *et al*. Antimicrobial activity of Argentine plants used in the treatment of infectious diseases. Isolation of active compounds from *Sebastiania brasiliensis*. J Ethnopharmacol 2001; 77: 37-40. http://dx.doi.org/10.1016/S0378-8741(01)00266-5

[10] Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. Science 1999; 284: 1318-22. http://dx.doi.org/10.1126/science.284.5418.1318

[11] Chmielewski RAN, Ftank JF. Inactivation of Listeria monocytogenes biofilms using chemical sanitizers and heat. In: Blaschek HP, Wang HH, Alge ME, Eds. Biofilms in the Food Environment. Iowa: Blackwell Publishing, 2007; pp. 73-104.

[12] Paz C, Cárcamo G, Silva M, Becerra J, Urrutia H, Sossa K. Drimendiol, a drimane sesquiterpene with quorum sensing inhibition activity. Nat Prod Comun 2013; 8: 147-8.

[13] Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet 2001; 358: 135-8. http://dx.doi.org/10.1016/S0140-6736(01)05321-1

[14] Sokol PA, Kooi C, Hodges RS, Cachia P, Woods DE. Immunization with a *Pseudomonas aeruginosa* elastase peptide reduces severity of experimental lung infections due to *P. aeruginosa* or *Burkholderia cepacia*. J Infect Dis 2000; 181: 1682-92. http://dx.doi.org/10.1086/315470

[15] O'Toole GA, Kolter R. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol Microbiol 1998; 30: 295-304. http://dx.doi.org/10.1046/j.1365-2958.1998.01062.x

[16] Caballero AR, Moreau JM, Engel LS, Marquart ME, Hill JM, O'Callaghan RJ. *Pseudomonas aeruginosa* protease IV enzyme assays and comparison to other *Pseudomonas proteases*. Anal Biochem 2001; 290: 330-7. http://dx.doi.org/10.1006/abio.2001.4999

[17] Mashimbye MJ, Maumela MC, Drewes SE. A drimane sesquiterpenoid lactone from *Warburgia salutaris*. Phytochemistry 1999; 51: 435-8. http://dx.doi.org/10.1016/S0031-9422(98)00753-5

[18] Asakawa Y, Toyota M, Oiso Y, Braggins JE. Occurrence of polygodial and 1-(2,4,6-trimethoxyphenyl)-but-2-en-1-one from some ferns and liverworts: Role of pungent components in bryophytes and pteridophytes evolution. Chem Pharm Bull 2001; 49: 1380-1. http://dx.doi.org/10.1248/cpb.49.1380

[19] Cortes MM, Oyarzun LM. Tadeonal and isotadeonal from *Drimys winteri*. Fitoterapia 1981; 52: 33-5.

[20] Fujita KI, Kubo I. Multifunctional action of antifungal polygodial against *Saccharomyces cerevisiae*: Involvement of pyrrole formation on cell surface in antifungal action. Bioorg Med Chem 2005; 13: 6742-7. http://dx.doi.org/10.1016/j.bmc.2005.07.023

[21] Bastos JK, Kaplan MAC, Gottlieb OR. Drimane-Type Sesquiterpenoids as Chemosystematic Markers of Canellaceae. J Brazil Chem Soc 1999; 10.

[22] Derita MG, Leiva ML, Zacchino SA. Influence of plant part, season of collection and content of the main active constituent, on the antifungal properties of *Polygonum acuminatum* Kunth. J Ethnopharmacol 2009; 124: 377-83. http://dx.doi.org/10.1016/j.jep.2009.05.029

[23] Taniguchi M, Adachi T, Oi S, Kimura A, Katsumara S, Isoe S, *et al*. Structure-Activity Relationship of the *Warburgia* Sesquiterpene Dialdehydes. Agr Biol Chem Tokyo 1984; 48: 73-8. http://dx.doi.org/10.1271/bbb1961.48.73

[24] Lunde CS, Kubo I. Effect of Polygodial on the Mitochondrial ATPase of *Saccharomyces cerevisiae*. Antimicrob Agents Chemother 2000; 44: 1943-53. http://dx.doi.org/10.1128/AAC.44.7.1943-1953.2000

[25] Song JH, Yang TC, Chang KW, Han SK, Yi HK, Jeon JG. *In vitro* effects of a fraction separated from *Polygonum cuspidatum* root on the viability, in suspension and biofilms, and biofilm formation of mutans streptococci. J Ethnopharmacol 2007; 112: 419-25. http://dx.doi.org/10.1016/j.jep.2007.03.036

[26] Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999; 12: 564-82.

[27] Smith JE, Tucker D, Watson K, Jones GL. Identification of antibacterial constituents from the indigenous Australian medicinal plant *Eremophila duttonii* F. Muell. (Myoporaceae). J Ethnopharmacol 2007; 112: 386-93. http://dx.doi.org/10.1016/j.jep.2007.03.031

[28] Sandasi M, Leonard CM, van Vuuren SF, Viljoen AM. Peppermint (*Mentha piperita*) inhibits microbial biofilms *in vitro*. S Afr J Bot 2011; 77: 80-5. http://dx.doi.org/10.1016/j.sajb.2010.05.011

[29] Gilabert M, Ramos AN, Schiavone MM, Arena ME, Bardón A. Bioactive sesqui- and diterpenoids from the argentine liverwort *Porella chilensis*. J Nat Prod 2011; 74: 574-9. http://dx.doi.org/10.1021/np100472d

[30] Anke H, Stern O. Comparison of the antimicrobial and cytotoxic activities of twenty unsaturated sesquiterpene dialdehydes from plants and mushrooms. Planta Med 1991; 57: 344-6. http://dx.doi.org/10.1055/s-2006-960114

[31] Eneroth A, Ahrne S, Molin G. Contamination routes of Gram-negative spoilage bacteria in the production of pasteurised milk, evaluated by randomly amplified polymorphic DNA (RAPD). International Dairy Journal 2000; 10: 325-31. http://dx.doi.org/10.1016/S0958-6946(00)00055-8

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