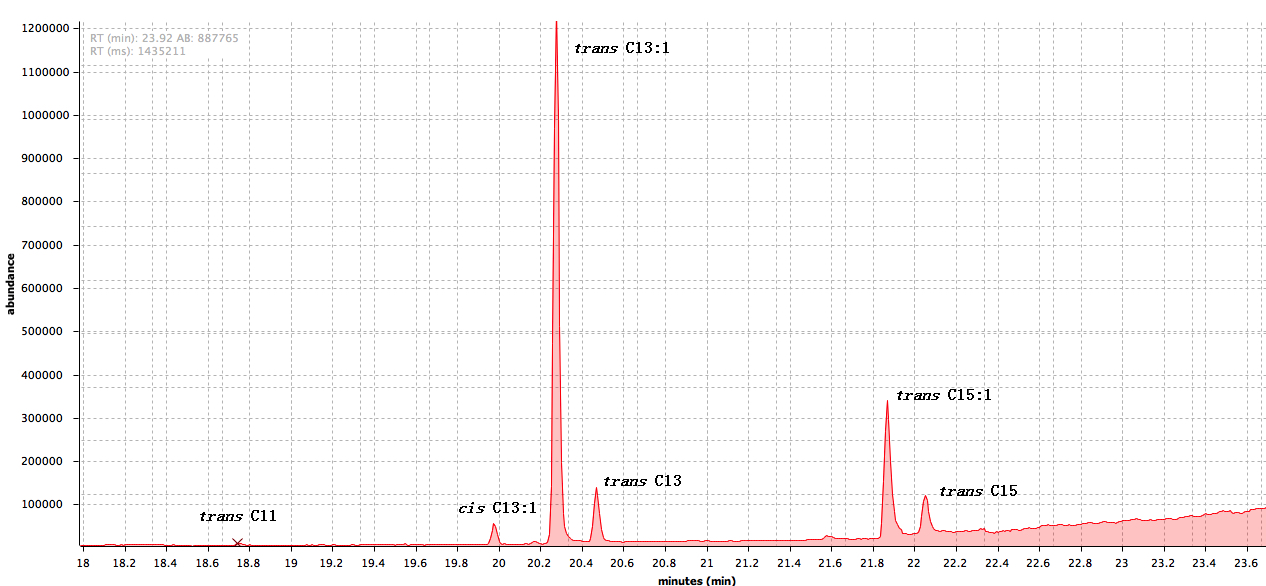
Supplementary Materials: Fire Ant Venom Alkaloids Inhibit Biofilm Formation

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**Figure S1.** Representative chromatogram of venom alkaloids extracted with hexane: acetone elution as described in [32], with peaks identified as described in [34], from red imported fire ants *Solenopsis invicta* collected in Rio de Janeiro, Brazil.

C:\Users\lenovo\Desktop\FigureS1_toxins.tiff

**Figure S2.** Linear regression from mean obtained inhibition halos from disk-diffusion using different concentrations (μg/mL) of solenopsins added to a confluent *Pseudomonas fluorescens* growth plate, incubated at 25 °C for 24 h. Same results as presented in Figure 1, herein transformed for MIC estimation (at x = 0, where indicated) according with [53].

**Table 1.** Viability of cells recovered from 1 mL of biofilm of *Pseudomonas fluorescens* formed on surfaces of polystyrene or stainless steel conditioned with venom solenopsins extracted from the red imported fire ant *Solenopsis invicta*.

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Polystyrene** | **Stainless Steel** |
| **Viable Cells** | **Viable Cells** |
| Control  0 µg/mL | 130.00 ± 26.53 *a* | 155.00 ± 35.16 |
| 1000 µg/mL | 36.00 ± 16.38 *b* | Not detected |
| 5,000 µg /mL | 37.00 ± 7.31 *b* | Not detected |

Means followed by SD (*N* = 3); different letters within the same column indicate statistically different values by Kruskal-Wallis followed by Dunn’s test at alpha = 0.05; values between rows (materials) did not differ by Wilcoxon’s rank test at alpha = 0.05 (W = 105, p-value = 0.7714).

**Table S2.** Values of surfactants and their respective applications (Adapted from [54]).

|  |  |
| --- | --- |
| **HLB scale** | **Use** |
| 4-6 | W/O emulsifiers |
| 7-9 | Wetting agents |
| 8-18 | O/W emulsifiers |
| 13-15 | Detergents |
| 15-18 | Solubilizers |

Notes: HLB - Hydrophilic-lipophilic balance; W - water; O – oil.

**Table S3.** Physicochemical properties of surfaces conditioned with rhamnolipids extracted from *Pseudomonas* *aeruginosa* and venom solenopsins extracted from red imported fire ants *Solenopsis invicta.*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Surface** | **Treatment** | **ƟW(degrees)** | **ΔGiwi (mJ/m2)** | **Ɣ LW (mJ/m2)** | **Ɣ AB (mJ/m2)** | **Ɣ + (mJ/m2)** | **Ɣ − (mJ/m2)** |
| Polystyrene | Control (H2O) | 66.7 ± 0.3 | −51.0 ± 1.7 | 46.9 ± 1.7 | 6.9 ± 0.5 | 3.5 ± 0.3 | 3.5 ± 0.3 |
|  | RL | 10.7 ± 1.2 | −30.0 ± 0.5 | 79.2 ± 0.2 | 18.4 ± 0.1 | 9.2 ± 0.1 | 9.2 ± 0.1 |
|  | Control (EtOH) | 64.4 ± 0.9 | −57.4 ± 0.8 | 50.1 ± 0.7 | 5.5 ± 0.0 | 2.7 ± 0.0 | 2.7 ± 0.0 |
|  | Sol | 63.3 ± 1.4 | −24.8 ± 0.7 | 51.6 ± 0.6 | 21.1 ± 0.6 | 10.6 ± 0.3 | 10.6 ± 0.3 |
| Stainless Steel 304 | Control (H2O) | 74.3 ± 0.8 | −76.0 ± 5.1 | 58.0 ± 1.6 | 2.3 ± 0.4 | 1.1 ± 0.1 | 1.1 ± 0.2 |
|  | RL | 7.9 ± 0.6 | −32.5 ± 1.3 | 80.7 ± 0.6 | 18.2 ± 0.2 | 9.1 ± 0.1 | 9.1 ± 0.1 |
|  | Control (EtOH) | 60.5 ± 0.3 | −58.0 ± 0.5 | 51.5 ± 0.4 | 5.2 ± 0.1 | 2.6 ± 0.1 | 2.6 ± 0.1 |
|  | Sol | 62.7 ± 1.7 | −58.4 ± 0.3 | 59.8 ± 0.2 | 5.6 ± 0.7 | 2.8 ± 0.3 | 2.8 ± 0.3 |

Means followed by SD (*N* = 4); Water contact angle - ƟW; Surface hydrophobicity - ΔGLW; Lifshitz-van der Waals component - ƔLW; Lewis acid-basic properties - ƔAB; electron donor component - Ɣ-and electron acceptor component – Ɣ+; RL - Rhamnolipids; Sol –Solenopsin alkaloids.