

Evaluation of natural antimicrobial agents against *Pseudomonas syringae* pv. *actinidiae*

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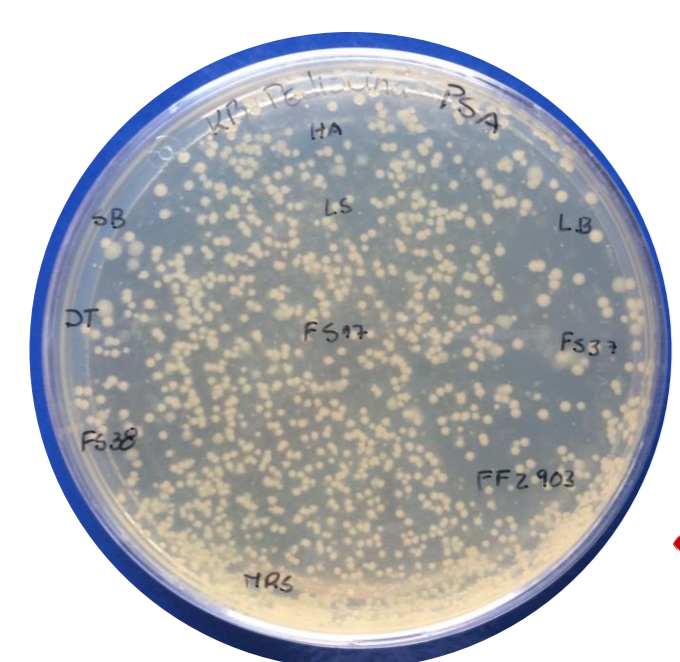
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Introduction

In Portugal the production of green and yellow kiwifruits is ca. 25.000 tones, representing an important source of income; however, these species are susceptible to *Pseudomonas syringae* pv. *actinidiae* (PSA), a gram-negative bacteria responsible for the kiwifruit bacterial canker. PSA was identified in Portugal in 2011, leading to production losses of up to 80 % in several orchards.

As current control techniques are expensive and consist in the application of copper- and antibiotics-based compounds, with serious risks of environmental contamination and development of resistant bacteria, the aim of this work was to evaluate the antimicrobial potential of natural origin compounds against PSA.

Results and Discussion



Bacteriocins showed **no significant** activity against PSA, probably because they are more effective against **Gram-positive** bacteria.

Propolis and **chitosan** showed the **highest inhibitory** ability in agar plate, with minimum inhibitory concentrations as low as 0.075 % and 0.25 %, respectively. This could have been due to **severe cell damage** through disturbances in membrane functions, changes in intracellular structures and cell wall fraying and weakening.

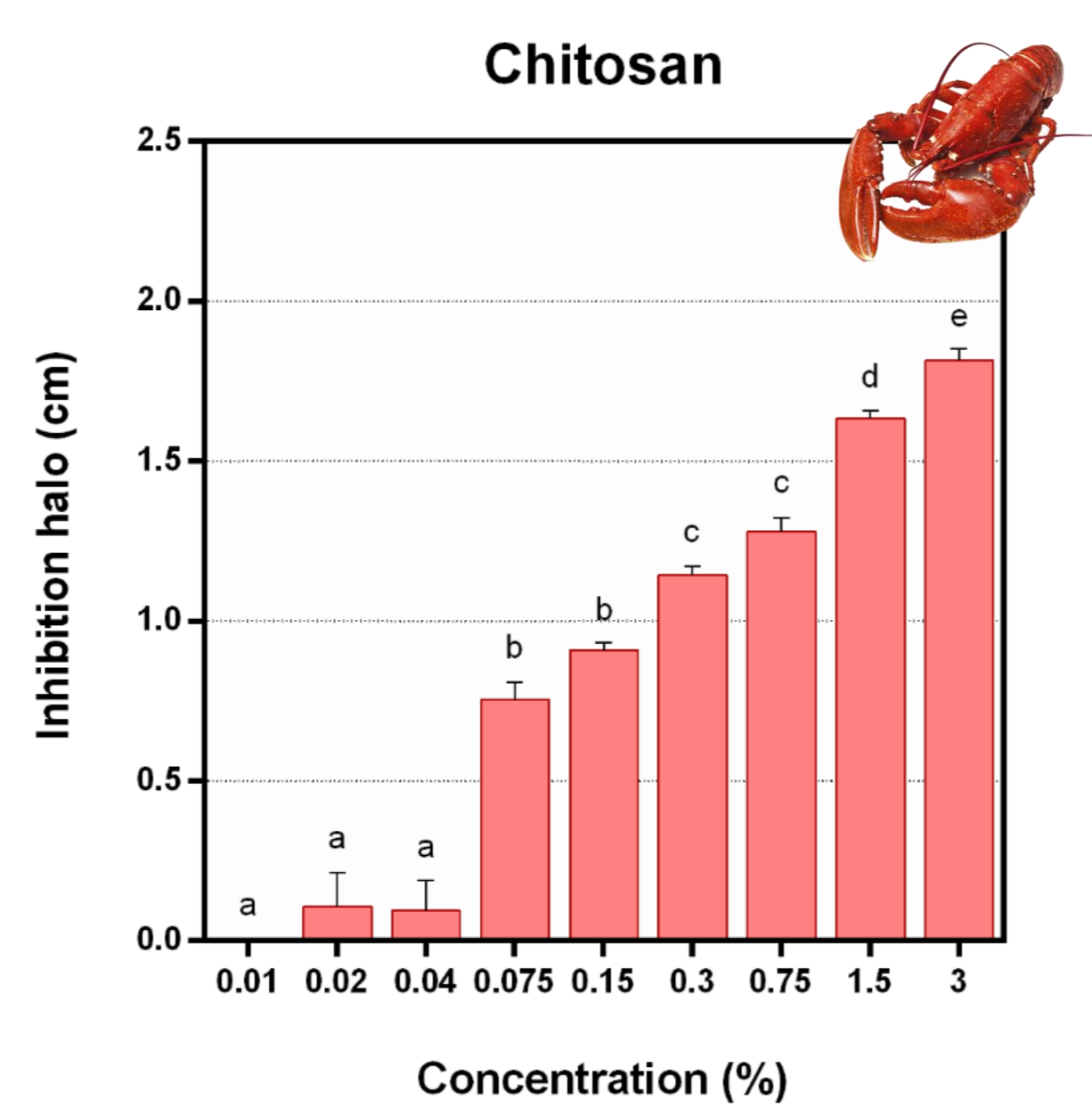
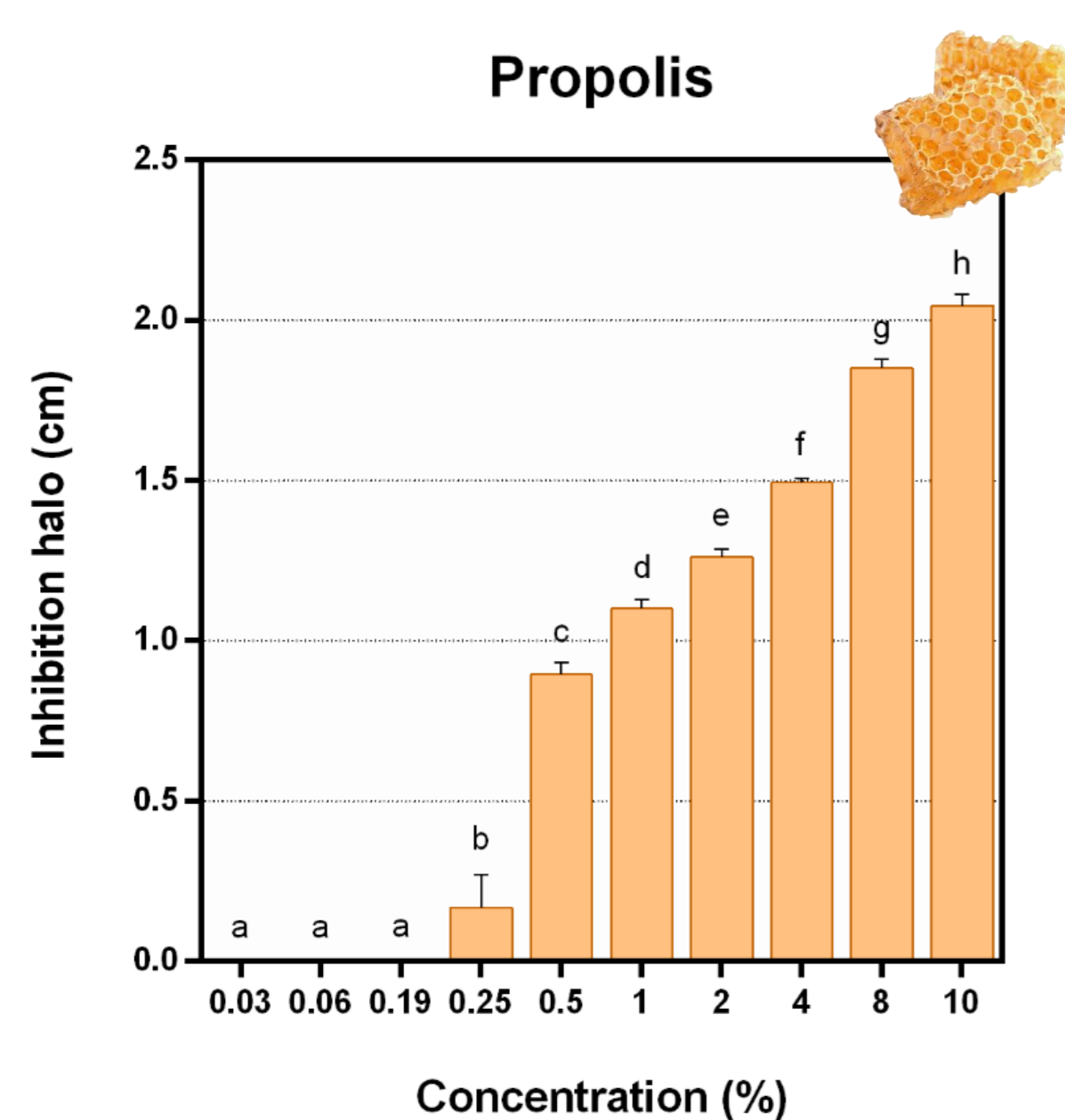


Table 1 and 2 - Colony Forming Units (CFUs) estimated in NSA plates incubated at 28 °C for 48 h cultured with PSA inoculum after treatment with different concentrations of propolis and chitosan (respectively) during 5 min, 1 h, 5h and 24 h. Key: (-) 0 CFUs; (+) 1 to 50 CFUs; (++) 51 to 100 CFUs; (+++) 101 to 300 CFUs; (++++) > 301 CFUs.

| Propolis Concentration | Time | | | | Chitosan Concentration | Time | | | |
|------------------------|-------|------|------|------|------------------------|-------|------|------|------|
| | 5 min | 1 h | 5 h | 24 h | | 5 min | 1 h | 5 h | 24 h |
| 0.5 % | ++ | ++ | ++ | ++ | 0.15 % | - | - | - | - |
| 1 % | ++ | ++ | ++ | ++ | 0.3 % | - | - | - | - |
| 2 % | ++ | ++ | ++ | ++ | 0.6 % | - | - | - | - |
| Control | ++++ | ++++ | ++++ | ++++ | Control | ++++ | ++++ | ++++ | ++++ |

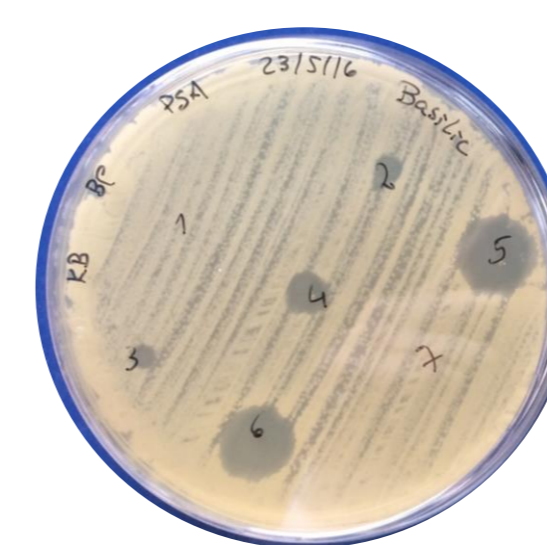
Chitosan inhibited PSA growth in liquid media after **only 5 minutes** of exposure with concentrations as low as 0.15 %.

Methods

Test compounds

- ❖ **Bacteriocins** (pediocin, sakacin, buchenericin and nisin): polycyclic antibacterial peptides produced by lactic acid bacteria
- ❖ **Propolis**: resinous substance obtained from bee hives
- ❖ **Chitosan**: polysaccharide derivative of chitin, found in the exoskeleton of crustaceans
- ❖ **Plant essential oils** (anise, basil, cardamom, fennel and laurel): concentrated hydrophobic liquid containing volatile compounds

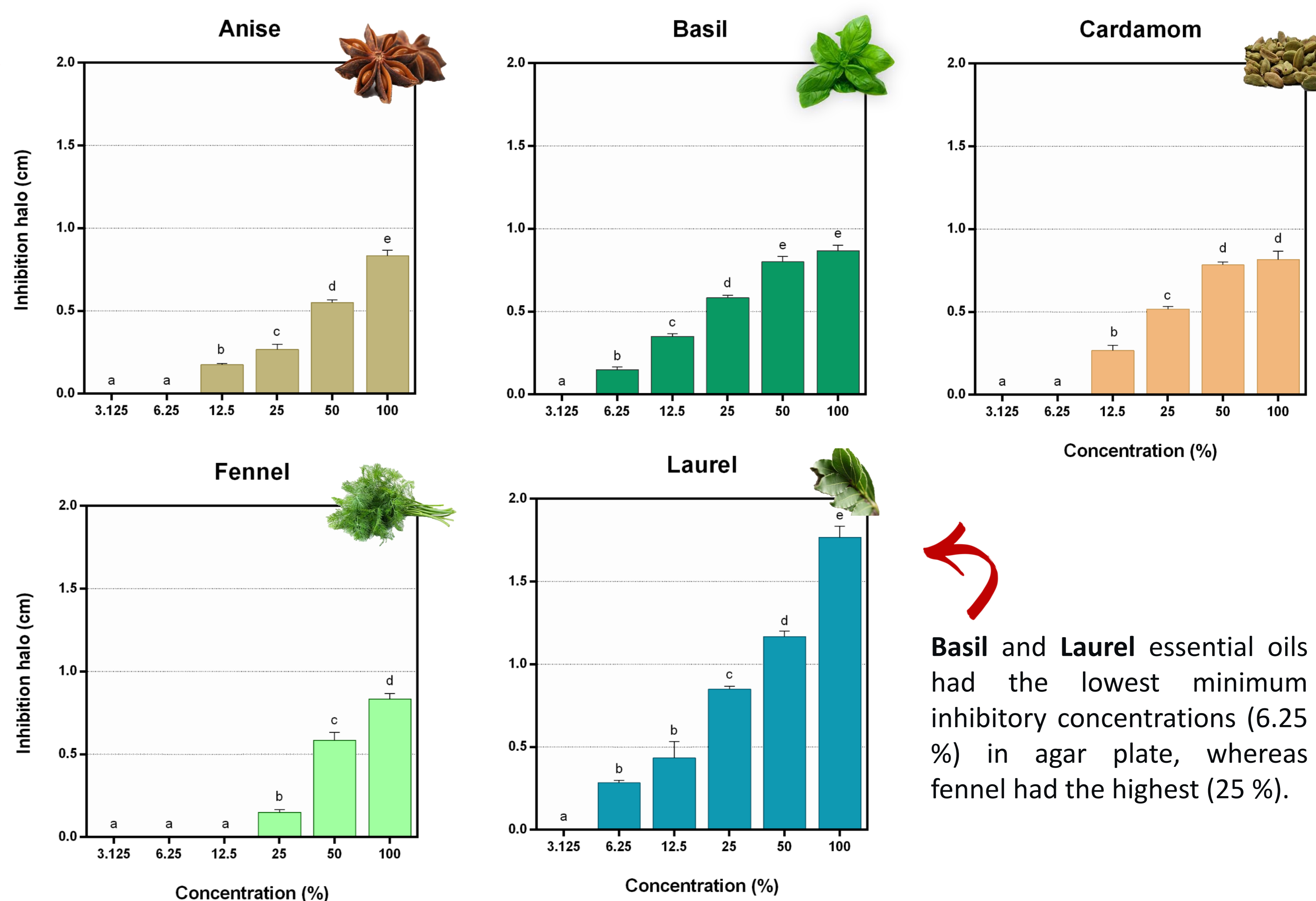
PSA inhibition in agar plates



1. PSA inoculum was plated onto King's B medium,
2. 10 µL droplets of several concentrations of each test compound were placed equidistantly in 3 different plates (3 replicates),
3. Plates were incubated at 28 °C for 48 h, after which the inhibition zone (halo) was measured.

PSA inhibition in liquid media

1. PSA inoculum was prepared in LB medium,
2. Equal volumes of inoculum and test compound solution were mixed and incubated for 5 min, 1 h, 5 h and 24 h at 28 °C for 48 h,
3. After each incubation time, 100 µL of each mixture were plated in NSA medium and kept at 28 °C for 48, after which PSA growth was evaluated.



Basil and **Laurel** essential oils had the lowest minimum inhibitory concentrations (6.25 %) in agar plate, whereas fennel had the highest (25 %).

Laurel essential oils showed the **fastest inhibitory reaction** in liquid media, impairing PSA growth after **only 5 min** of exposure, possibly because it is mainly composed by 1,8-cineole, which was already shown to have great antibacterial activity

Table 3 - Colony Forming Units (CFUs) estimated in NSA plates incubated at 28 °C for 48 h cultured with PSA inoculum after treatment with different concentrations of five essential oils (anise, cardamom, basil, fennel and laurel) during 5 min, 1 h, 5h and 24 h. Key: (-) 0 CFUs; (+) 1 to 50 CFUs; (++) 51 to 100 CFUs; (+++) 101 to 300 CFUs; (++++) > 301 CFUs; nt: not tested.

| Concentration | Anise | | | | Cardamom | | | | Basil | | | | Fennel | | | | Laurel | | | |
|---------------|-------|-----|-----|-----|----------|-----|-----|-----|-------|-----|-----|-----|--------|-----|-----|-----|--------|-----|-----|-----|
| | 5 min | 1h | 5h | 24h | 5 min | 1h | 5h | 24h | 5 min | 1h | 5h | 24h | 5 min | 1h | 5h | 24h | 5 min | 1h | 5h | 24h |
| 6.25 % | nt | nt | nt | nt | nt | nt | nt | nt | ++++ | +++ | +++ | +++ | nt | nt | nt | nt | - | - | - | - |
| 12.5 % | +++ | +++ | +++ | +++ | +++ | +++ | ++ | ++ | +++ | + | + | + | nt | nt | nt | nt | - | - | - | - |
| 25 % | +++ | +++ | +++ | +++ | - | - | - | - | ++ | - | - | - | +++ | +++ | +++ | +++ | - | - | - | - |
| 50 % | +++ | +++ | +++ | +++ | - | - | - | - | nt | nt | nt | nt | + | - | - | - | nt | nt | nt | nt |
| 100 % | nt | nt | nt | nt | nt | nt | nt | nt | nt | nt | nt | nt | + | - | - | - | nt | nt | nt | nt |
| Control | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |

Conclusions

- ✓ All compounds, except bacteriocins, had a significant antimicrobial activity against PSA in agar plates after 48 h of exposure.
- ✓ Chitosan and laurel essential oil showed total PSA inhibition in liquid media after only 5 min of exposure.
- ✓ Results provide strong evidence on the potential use of natural compounds in the formulation of more environmentally friendly products against PSA.