A comparative analysis of the antibiofilm properties of crude blueberry extracts against MRSA and MSSA

Silva S.[1*]; Costa E.M. [1]; Costa M.R.[1]; Pereira M.F. [1]; Morais R.M. [1]; Pintado M.E. [1]

[1]*CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072-Porto, Portugal*
**snsilva@porto.ucp.pt*

BIOFILMS 5 International Conference

Introduction

Staphylococcus aureus is a known problem due to their vast array of virulence factors and growing antibiotic resistance, particularly so considering its capacity to adhere and survive in several surfaces (*e.g.* prosthetic devices**).** Taking this into account, a considerable effort has been employed in the search for new antimicrobial compounds that may help to prevent biofilm formation in surfaces, thus reducing the incidence of staphylococcal infections (Chambers 2001; Mann 2008; Rhode 2007).

Vaccinium corymbosum, also known as highbush blueberry, is one of the several species that produce blueberries, fruits known as a good source of phenolics, compounds with a known health promoting activity (namely antimicrobial properties) (Riihinen *et al*., 2008).

Given the recognized action of blueberry juice upon the prevention and treatment of urinary tract infections, due to their capacity to hamper cellular adhesion (thought to be a consequence of both anthocyanidins and mannose analogs present in the blueberries) (Burdulis *et al*., 2009; Cowan, 1999), the present work aimed to characterize the antibiofilm properties of several blueberry fruit and leaf extracts against both Methicillin-resistant *Staphylococcus aureus* (MRSA) and Methicillin-sensitive *Staphylococcus aureus* (MSSA).

Procedure

Powdered dry fruit and leaf

Infused extracts
- Fruit: 5 mg of fruit infused in 100 mL of boiling water for 15 min
- Leaf: 2 mg of fruit infused in 100 mL of boiling water for 45 min

Boiled extracts
- Fruit: 5 mg of fruit were boiled in 100 mL of water for 15 min
- Leaf: 2 mg of fruit were boiled in 100 mL of water for 30 min

Depectinization
(addition of pectinases from *Aspergillus aculeatus* (1% (V/V), incubation for 1 h at 40 °C, heating to 100 °C and filtering)

MIC Determination
MRSA; MSSA and *Chromobacterium violaceum* (*C. violaceum*)

Biofilm inhibition
- Total viable counts determined after a 48 h incubation period in contact with the extracts.
- Results calculated as the logaritmic reduction of the total viable cells.

Quorum-sensing
- Screening for ihnibition of Quorum sensing was performed by assaying the production of violecein in the presence of the extracts.

Results

Table 1 – Minimum inhibitory concentration, in mg/mL, for all the extracts against MRSA and MSSA.

	Fruit infusion	Boiled fruit	Leaf infusion	Boiled leaf
MRSA	50	50	12.5	12.5
MSSA	50	50	12.5	12.5
C. violaceum	50	50	25	25

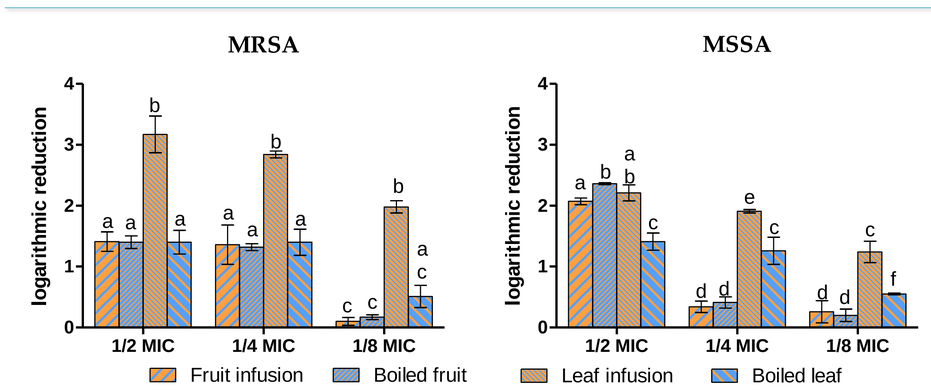

Figure 1 – Logarithmic reduction of the total viable counts in biofilms formed in the presence of half (1/2), one fourth (1/4) and one eight (1/8) of the MIC for both MRSA and MSSA.

Table 2 – Inhibition of violecein production by *C. violaceum* when in the presence of the extracts at one half (1/2), one fourth (1/4) and one eight (1/8) of the MIC. (-) absence of violecein; (+) production of violecein.

		Fruit infusion	Boiled fruit	Leaf infusion	Boiled leaf
Violecein production	½ MIC	-	-	-	-
	¼ MIC	+	+	-	-
	⅛ MIC	+	+	+	+

Conclusions

- All extracts exhibited some impact upon biofilm formation despite the use of sub-MICs.
- Leaf extracts appeared to possess a higher activity than blueberry fruit extracts, except for ½ of the MIC in MSSA.
- All extracts impacted upon the production of violecein, possibly hinting to some interference in intercellular communication.

References

- Burdulis, D., Sarkinas, A., Jasutiené, I., Stackevicené, E. Nikolajevas, L., Janulis, V. (2009) *Natural Drugs* **66**(4):399-408
- Chambers, H. F. (2001) *Emerging Infectious Diseases* **7**(2):178-182
- Cowan, M. M. (1999) *Clinical Microbiology Reviews* **12**(4):564-582
- Mann, N. H. (2008) *Research in Microbiology* **159**(5):400-405
- Rhode, T. M. (2007) *International Journal of Food Microbiology* **166**(3):372-383
- Riihinen, K., Jaakola, L., Karenlampi, S., Hohtola, A. (2008) *Food Chemistry* **56**(1):3-12

Acknowledgements

The authors hereby acknowledge the Agency of Innovation (Agência de Inovação, ADI, Portugal) and Quadro de Referência Estratégico Nacional (QREN, Portugal) which through the project “Myrtillus – Mirtilo com inovação” (QREN-ADI 13736) provided funding for the realization of this work, to the project PEst-OE/EQB/LA0016/2011 and Frulact S.A. For also supporting this work.