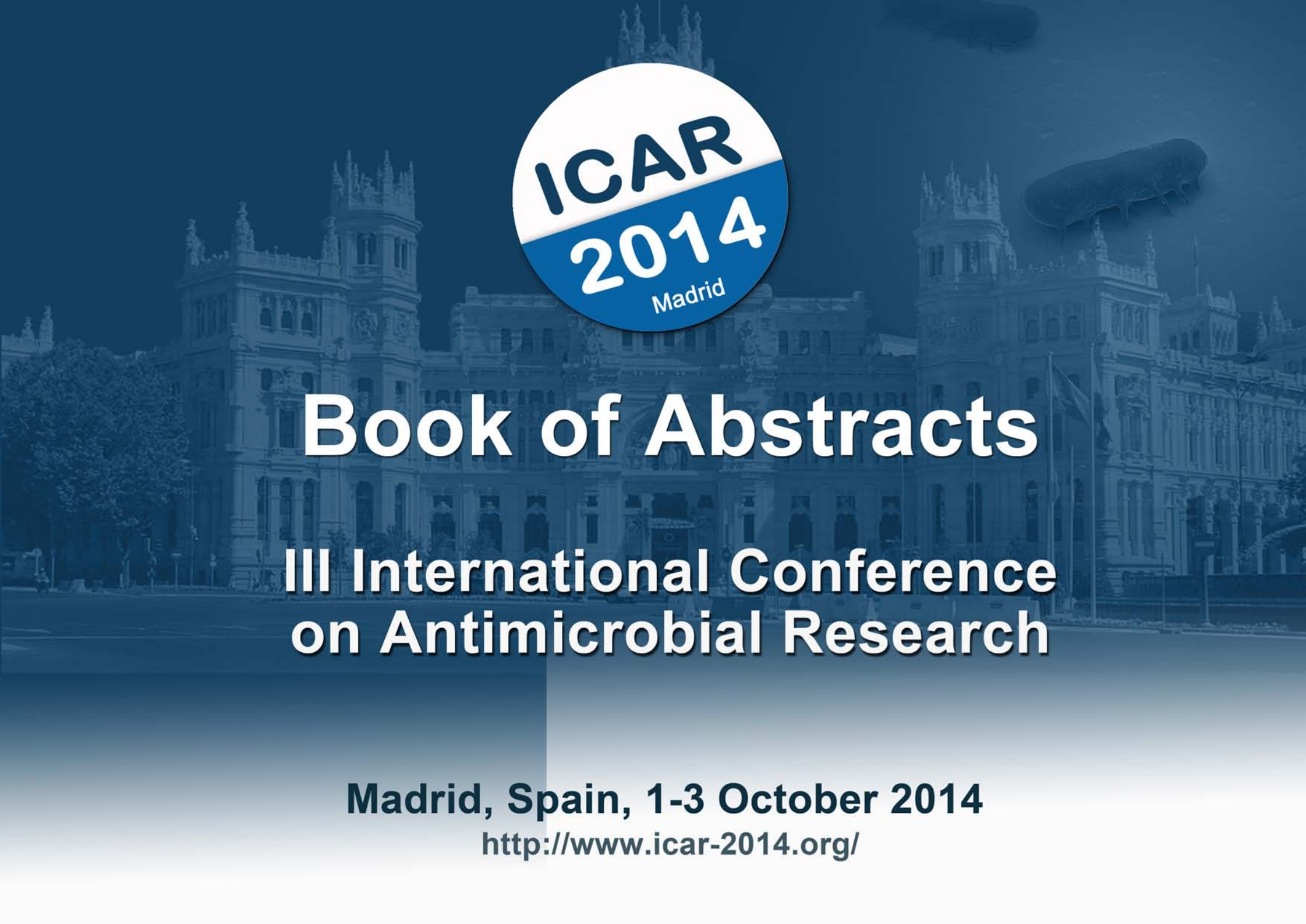

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Antimicrobial activity of an anthocyanin rich blueberry extract, purified using SPE

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There is a wide recognition of the biological potential of phenolic compounds, with their potential as antimicrobials gaining a particular interest when taking into account the emergence of microbial resistance and the need for alternative sources of antimicrobial compounds. Anthocyanins are one of the families of flavonoids that gather most of the attention for their biological potential therefore they are one of the main focus whenever blueberry extracts are concerned. As such, the present work aimed to evaluate the antimicrobial potential of a purified anthocyanin fraction (ca. 85% of total phenolics are anthocyanins) against a plethora of microorganims: multiresistant clinical isolates of *Escherichia coli*, *Proteus mirabilis*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, a Methicillin resistant (MRSA) and a methicillin sensitive (MSSA) *Staphylococcus aureus*, *Pseudomonas aeruginosa* 10145, *Escherichia coli* 25922 and MSSA ATCC 25923.

Firstly, the antimicrobial activity of the extract against each microorganism was screened by drawing inactivation curves. The analysis of these results showed that the most sensitive microorganisms was *S. aureus,* for which all strains studied were unable to grow in the presence of 500 μg/mL of powdered extract. *P. mirabilis* and *A. baumanii* suffered no inhibition. All other microorganisms, while not fully inhibited showed some signs of biological activity by the extract with delayed start of the exponential stage, lower growth rates and/or lower microbial loads after 24 and 48h (when compared to the control). As an example, the *E. coli* clinical isolate registered a microbial load 22 to 25% (after 24 and 48 h respectively) lower than the positive control when incubated with 500 μg/mL of powdered extract, while the collection strain registered reductions of 47 to 69% (after 24 and 48 h respectively) when under the same conditions. As for *P. aeruginosa* both strains portrayed longer *lag* stages when exposed to 500 μg/mL, with the clinical isolate exhibiting a 9 h longer lag stage than that of the control.

Secondly, the extracts capacity to inhibit biofilm formation was screened. In this test, all microorganims except *P. mirabilis* demonstrated some sensitivity toward the extracts, with inhibition percentages ranging from 75 to 45% when exposed to 500 μg/mL, 75-23% when exposed to a 250 μg/mL concentration and in some cases, the extracts remained active when used in concentrations as low as 50 μg/mL (35-21%). In this assay, the collection strain of *P. aeruginosa* appeared to be the most sensitive to the extracts activity with concentrations as low as 50 μg/mL still being able to cause an inhibition of 47 ± 12 % of biomass. It is interesting to note that, while the growth of *A. baumanii* was not affected by the extracts its biofilm formation was with a range of inhibition percentages from 74 to 35 %. For *P. mirabilis*, the presence of the extracts appears to strongly promote biofilm formation. Additionally, the extracts capacity to inhibit short term adhesion, a 3h adhesion test was performed with only the clinical isolates of *S. aureus* and the collection strain of *P. aeruginosa* exhibiting inhibition of bacterial adherence in this time frame.

Overall, the present work demostrated that anthocyanin rich fractions possess significant antimicrobial and antibiofilm activity against several multiresistant and culture collection microorganisms, thus showing their potential as possible active ingredients in future pharmacological solutions for the treatment of bacterial infections.

Keywords: anthocyanins; antibiofilm; antimicrobial; adhesion