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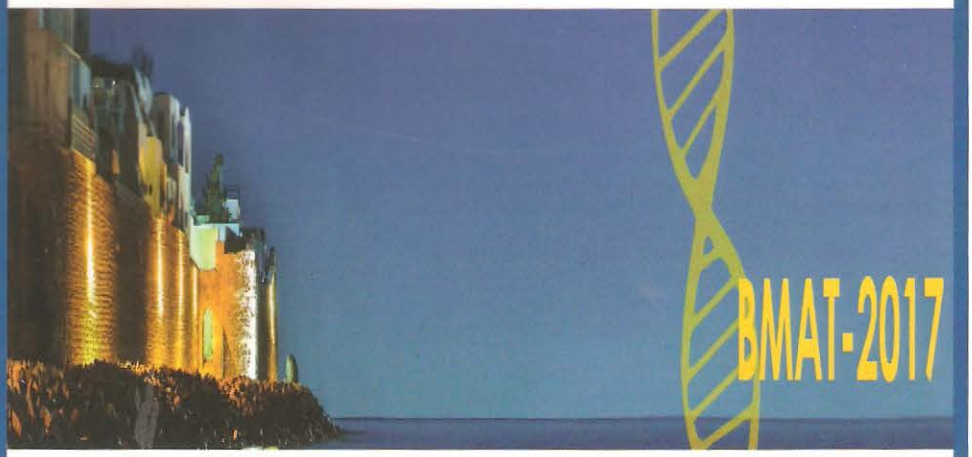

Biochemistry & Microbiology Applied Technologies

International Congress

November 3-5, 2017- Hammamet, Tunisia

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B-P-35

Antimicrobial activity of polyphenols extracted from winery by-products against drug resistant bacteria

Vanessa Silva[1], Gilberto Igrejas[2,3,4], Virgílio Falco[5], Joana Amaral[6,7],Carmen Torres[8], Patrícia Poeta[1,4]

[1]Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal;
[2]Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal;
[3]Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal;
[4]Research Unit on Applied Molecular Biosciences (UCIBIO-REQUIMTE), University Nova of Lisboa, Lisboa, Caparica, Portugal;
[5]Centro de Química-Vila Real (CQ-VR), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal;
[6]ESTiG, Polytechnic Institute of Bragança, Portugal;
[7]REQUIMTE-LAQV, Department of Chemical Sciences, Pharmacy Faculty, University of Porto, Portugal;
[8]Área de Bioquímica y Biología Molecular, Universidad de La Rioja, Logroño, La Rioja, Spain.

Background: The emergence of drug resistant bacteria worldwide is becoming a threat to human health since commonly used antibiotics are no longer effective against these bacteria. Therefore, it is necessary to investigate and develop new effective antimicrobials. Polyphenols are secondary metabolites of plants which confer health protective benefits. Several studies have demonstrated the antimicrobial activity of these compounds since they cause structural or functional damage to the bacterial cell membrane. Agro-industrial by-products, namely from the winery industry, are a potential source of polyphenols and can be used as cheap source of these compounds. Thus, the aim of this study was to extract the polyphenols from the winery by-products and evaluate their antimicrobial activity against antibiotic resistant bacteria.

Materials/Methods: Winery by-products, namely the seeds, peels and stems of two Portuguese varieties,'Touriga Nacional' and 'Preto Martinho', were freeze-dried and mill-powdered. The extraction of polyphenols was performed with water/ethanol (50:50, v/v) mixture. Antimicrobial susceptibility was tested against 10 different bacterial species: *E. faecalis*vanB2-C3735, *E. faecium*vanA-C2302, *E. coli* C999(CTX-M-15), *K. pneumoniae* C1370(CTX-M-15), *P. aeruginosa* C4660(VIM-2), *S. aureus* C5932(MRSA CC398), *S. epidermidis* C3658(linezo-R), *S. enteritidis* C4220, *L. monocytogenes* ATCC700302and *B. cereus*ATCC1306. Minimum inhibitory concentrations (MICs) were determined by agar dilution assay using different concentrations of polyphenol extracts. The evaluation of polyphenolic compounds in the extracts was achieved by High Performance Liquid Chromatography (HPLC) equipped with a UV-vis diode array detector.

Results: The seeds' extracts from both varieties showed the highest antimicrobial activity, as evidenced by the inhibitory effect on 7 out of the 10 bacterial strains tested. The seeds' extracts from Preto Martinho showed better results than 'Touriga Nacional'. The antimicrobial activity of the peels' extracts was higher for 'Touriga Nacional' whereas the stems' extracts from 'Preto Martinho'showed a higher inhibitory effect than 'Touriga Nacional'. There was no inhibitory effect of neither of the extracts on *S. enteritidis, E. coli* and *P. aeruginosa*.

Conclusion: All gram-positive bacteria showed susceptibility to polyphenols extracted from winery by-products. From the gram-negative bacteria tested only *K. pneumoniae* showed susceptibility to the seeds' extracts.